ON HOW A FROG IS NOT A CAT

by

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ABSTRACT

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A preparation and technique have been developed which permit intracellular recording from spinal cord motoneurons in the intact, curarized frog. This two-part investigation of synaptic activity in hindlimb motoneurons was concerned with: 1) elucidating the mechanisms and integrative properties of dendritic synapses, and, 2) demonstrating, through input-output relations, the relative roles of cutaneous sense and muscle sense in determining frog motor function.

In Part I various antidromic signals, especially the M spike, were interacted with the distal dendritic monosynaptic EPSP's in an attempt to find a measure sensitive to remote membrane resistance transients proposed to produce synaptic activity. In general no shunting of the M spike was found. A mathematical model of the neuron was developed to simulate the effect of dendritic membrane resistance transients on the voltage response to a current source acting from the cell body. Calculations based upon the model indicate that the degree of shunting in the experiments would be about 1%, well below the detection threshold for the M spike technique. It is concluded that whatever their synaptic mechanism, the EPSP's of distal dendritic origin appear to the cell body as if they are generated by essentially a current source. The implication of this situation for the combining of somatic and dendritic synaptic activity are discussed in terms of interaction effects dependent upon resistance and voltage transients.

It was found that the muscle afferents produce only monosynaptic dendritic EPSP's in frog motoneurons and that these EPSP's alone cannot bring about motoneuronal discharge. The general absence of a stretch reflex in frog is confirmed at the synaptic level.

In Part II it was found, using electrical stimulation of cutaneous and muscle nerves, that, in the intact frog, a stimulation rate of 60/min generally resulted in the abolition of all polysynaptic activity in motoneurons. At a stimulation rate of 6/min polysynaptic activity was very evident. In chronically spinalized animals this frequency-dependent effect was not evident. It is concluded that this effect is due to a descending inhibition acting on interneurons.

Electrical stimulation of cutaneous nerves even at 6/min was generally ineffective in eliciting motoneuronal discharge, but "natural" stimulation by light punctate touch was extremely effective. Touch to different skin areas resulted, in a given motoneuron, in PSP patterns that appeared as specific signatures of location. The roles of cutaneous and muscle sense in determining the posture and phasic motor activity in frog is discussed. It is concluded that the muscle activity in frog is governed far more by cutaneous sense than by muscle sense; this is in marked contrast to the situation prevailing in the cat where the muscle sense is considered to be dominant in determining motor activity. A possible relationship between the synaptic locus of the monosynaptic connections from muscle afferents and the existence of gravity-resistant postures is discussed. It is suggested, on electrophysiological grounds, that an enhanced ability to resist gravity and to perform precise motor acts can be associated with a movement of the synaptic terminals of the muscle afferents from the peripheral dendrites toward the cell body.

Thesis Supervisor: Jerome Y. Lettvin

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It is a pleasure to thank Professor Jerry Lettvin for his guidance and encouragement during the course of the work. Dr. Karl Kornacker's steady interest and ready advice were a firm support. Professor Tom Sheridan was understanding of my altered academic path. My wife, Diane, was patient, but persistent.

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INTRODUCTION

Frogs in their adult form represent aspects of the evolutionary transition from life in the water to life on the land. Certainly one interesting aspect of this transition is the development of a motor system that must now begin to control, in the presence of gravity, well-developed appendages to produce postures and locomotion appropriate to living on land. Frogs, whether on land or in water, spend a good deal of time waiting, either sitting or floating, while their senses, especially vision, report selectively on the environment. Their movement responses to food or predator is usually explosive and ballistic; locomotion is accomplished, in water or on land, either by a rapid dual extension of the rear legs, or by a slower quadrupedal gate.

Although much is known about the relatively sophisticated motor system of mammals, the motor system of the amphibian, possibly the prototype motor system for land life, has been little studied. This thesis is a study of the frog's motor system with attention focused on the input-output relations for spinal cord motoneurons. The study is divided into two parts. The first part is an attempt to utilize the specificity of location of certain monosynaptic motoneuron inputs in the frog to examine the mechanisms and integrative properties of dendritic synapses. The second part is a study of the relative importance of muscle sense versus cutaneous sense in determining the functional output of frog motoneurons. These two parts will be considered separately until the Discussion where it will be shown that they come together when one considers the motoneuron as an element in the system producing motor function.

Part I

Synaptic Mechanisms

At present, there are considered to be three general mechanisms by which the activity or information in one nerve cell can influence an adjoining nerve cell at their synapse. These three distinct mechanisms give rise to the following labeling of synapses--the chemical synapse, the electrical synapse, and the ion-pump synapse.

In the case of chemical synaptic transmission, the depolarization of the presynaptic axon terminals results in the liberation of a chemical substance (the transmitter) that diffuses across the synaptic cleft and interacts with the postsynaptic membrane to produce a brief (1-2 msec) increase in the permeability (conductance) of the local subsynaptic membrane to specific ions. The resultant movement of the ions down their respective electro-chemical gradients is a current that produces transient potential changes across the postsynaptic membrane (the postsynaptic potential, or PSP). In the case of electrical synaptic transmission, the presynaptic and postsynaptic membranes are tightly opposed, and the electrical resistance across the two membranes is small enough, relative to the extracellular resistance path, to permit a portion of the presynaptic current to flow directly into the postsynaptic cell. Thus, a PSP is produced without a conductance change in the local postsynaptic membrane. In the case of the ion-pump synapse, the transmitter is believed to alter the activity of the sodium-pump mechanism in the postsynaptic membrane (Pinsker and Kandel, 1969). This synaptic mechanism is associated with slow PSP's lasting 100 msec or more.

Prior to the first use of the intracellular microelectrode to record from nerve cells (Brock, Coombs and Eccles, 1962a; Araki, Otani and Furukawa,

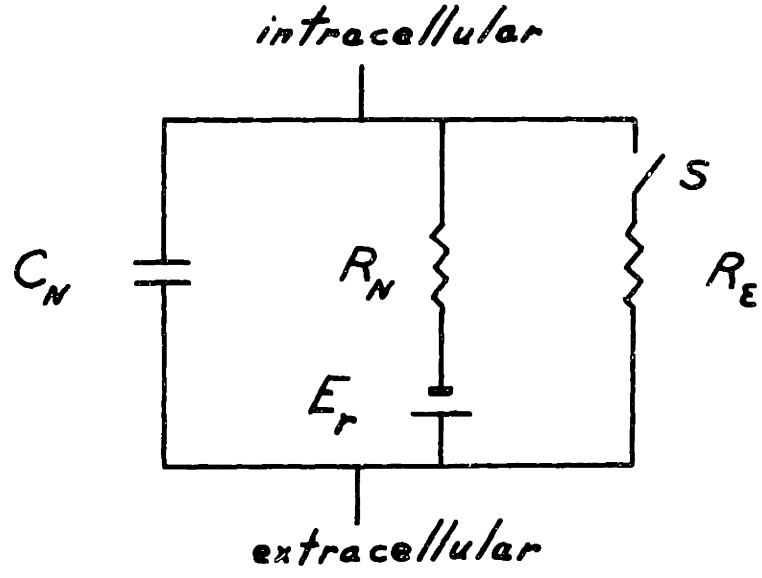
1953), the most favored explanations for synaptic activity were akin to the electrical mechanism (Eccles, 1950). Since that time, however, the stance has completely shifted toward emphasizing the chemical transmitter mechanism, especially for the higher vertebrates. However, in the frog, examples of each of the other two types of mechanisms are apparently present (Grinnell, 1966; Nishi and Koketsu, 1968, but see Kobayashi and Libet, 1968).

Certainly a main driving force for the adoption of the chemical synapse point of view was the extensive work done by Eccles and his collaborators on the synaptic activity of motoneurons in the spinal cord of the cat (Eccles, 1957, 1964a). Actually, the mechanism proposed by the Eccles group to account for the EPSP was an extension of the chemical transmitter mechanism presented by Fatt and Katz (1951) for the operation of the crayfish neuromuscular junction. In the beginning of the intracellular-electrode era, the preliminary explanation for the IPSP was that the chemical transmitter resulted in an increased activity of the sodium pump (Brock, Coombs and Eccles, 1951b); but after the work of Fatt and Katz (1953) on the inhibitory synapses of the crustacean muscle fiber, the Eccles group proposed that the IPSP also is produced by a conductance change, but by one which is specific to K^+ and/or to Cl^- ions (Coombs, Eccles and Fatt, 1955a).

Synaptic Location and Neuron Models

The electrical circuit proposed by the Eccles group to model the motoneuron and PSP generation therin is a condensation of two aspects of synaptic activity (see Fig. /). The first aspect is a statement about the synaptic mechanism, and the second aspect is an implicit statement about the relevancy of different synaptic loci. With regard to mechanism, the model indicates that PSP's are produced by the ionic currents resulting from brief transient membrane conductance changes for ions. With regard to

Figure 7. Circuit proposed to represent the postsynaptic membrane and the chemical synaptic mechanism. In addition to representing a portion of the neural membrane, this model has been commonly used to represent the entire motoneuron. An EPSP (excitatory postsynaptic potential) is simulated by a brief closure of switch S.


intracellular
S
C_N
R_N
R_E
E_r
extracellular

synaptic loci, the model implies that the synapses which are influential in impulse generation are located on the cell body and/or the large proximal dendrites. That is, a lumped parameter model of the neuron indicates that all relevant conductance transients are connected in parallel directly to the essentially isopotential cell body. There is no feature of the model that considers the electrotonic coupling, via dendritic core resistances, of synaptic events in the more distal dendrites to the cell body, and, hence, to the presumed site of spike initiation in the initial axonal segment. The ineffectiveness of synapses located on the more distal portions of the dendrites has also been explicitly stated by Eccles (1964b, 1960).

Several of the properties of the lumped parameter model, predominantly with respect to EPSP's, have not been consistently verified by various experimenters. According to the model, an increase in the membrane potential (hyperpolarization) should result in an increase in the magnitude of the EPSP. Most attempts to verify this proportionality between EPSP and membrane potential have revealed no change in the size of the EPSP with increased membrane potential (Coombs, Eccles, and Fatt, 1955b; Smith, Wuerker, and Frank, 1967). The model also indicates that there is a unique equilibrium potential for the EPSP and that further depolarization of the membrane should result in a reversal of sign of the EPSP. There are conflicting reports as to the existence of such behavior (Smith, et al., 1967). The behavior predicted by the model for IPSP's has been reasonably substantiated, but the model remains questionable for the case of EPSP's.

The model could be discrepant with experiments, because either the chemical-transmitter mechanism is not really so predominant in the vertebrate, or because no provisions were made to represent the consequences of synaptic location. Thus, there are two linked problems to consider; one,

the mechanism of the synaptic action, and two, the efficacy and, thus, the functionality of the more remote dendritic synapses. Customarily, one supposes that if hyperpolarization and depolarization at the cell body (soma) have no effect on the size or shape of an EPSP, then it is associated with a dendritic synaptic locus since it is assumed that the synaptic site is electrically too distant from the microelectrode in the soma for the injected current to produce a detectable effect. A negative finding of this type is more of a challenge to the simple lumped-parameter model of the neuron than it is to the nature of the synaptic mechanism. This is so because both the chemical transmitter mechanism and some electrical mechanisms would be similarly effected by the relatively small changes in membrane polarization that are produced in the dendrites. The need to consider synaptic loci in any relvant model of a neuron exists not only because of the confusion caused by trying to interpret experiments in terms of the lumped model, but also because of the anatomical findings that consistently show that the dendrites are as densely covered with synapses as is the cell body (Romanes, 1964; Sprague and Ha, 1964).

Models of a neuron that consider the effects of PSP's generated at different synaptic loci are due largely to Rall (1962, 1964, 1967). His first model was a purely distributed parameter model in which the conductance change mechanism was introduced into what is essentially a finite cable transmission line representation of the passive neuron. This analytical model was a 2-region representation which permitted simulation of PSP activity over any fraction of the neuron surface. His more recent model is designed to permit more selective localization of PSP activity; it is a quasi-distributed model in which the neuron is represented by a linkage of compartments, each of which is a lumped parameter representation of a portion of the neuron.

Interaction of Antidromic Signals with EPSP's

The first part of this thesis is concerned with the intertwined considerations of synaptic location and synaptic mechanism in representations of neurons. The consequences of synaptic location for the detectability of the existence of postsynaptic membrane conductance changes are investigated in frog motoneurons. The frog motoneuron was used because it provides for investigation monosynaptic EPSP's which have an exclusively dendritic origin (Fadiga and Brookhart, 1960). With these synapses attempts were made to find an indicator of dendritic conductance changes. The approach used involved modifications of the notion that the conductance transient change proposed to accompany an EPSP would shunt the development of antidromic spike invasion of the cell body. This approach has been used successfully by Fatt and Katz (1961) in the amphibian neuromuscular junction and by Kuffler and Eyzaquirre (1955) in the crustacean stretch receptor, but the expected reduction was not found by Coombs, Eccles and Fatt (1955b) in the cat motoneuron. The problem with using the full antidromic spike as an indicator or measure of synaptic conductance changes is that the spike invasion itself is an active process involving transient conductance changes. These changes, as seen by a somatically located microelectrode, are themselves so much larger than the changes associated with EPSP's, even those on the soma, that the latter changes are overshadowed, and the spike height changes little, if at all. In fact, a counteracting situation is present, because the EPSP depolarization enhances the antidromic invasion of the soma and tends to synchronize the active invasion process over the soma surface. This effect may lead to a larger

antidromic spike with the EPSP than without the EPSP. Another confounding factor regarding the use of the height of the full antidromic spike as an indicator of membrane conductance transients stems from the possible occurrence of sodium inactivation due to the EPSP depolarization. The presence of this effect would tend to result in a decrease in the height of the antidromic spike.

To circumvent the above problems an attempt was made to use the naturally occurring blockage of soma invasion to obtain as the measure a current source that would be independent of the voltage change associated with EPSP's. That is, the cell axon would be as a second, but naturally existing microelectrode presenting a current source to the soma-dendrite impedance. If this situation existed, then the peak height of the somatically recorded voltage transient associated with the current injection should be less when it occurred together with EPSP's than when it occurred alone. There are, however, several caveats to this approach. First, the portion of the current associated with frequencies of 1 kc and higher will pass across the membrane predominantly as capacitative current and also primarily across the soma membrane rather than along the dendritic core resistance and through the dendritic membrane. Since the capacitance of the membrane is taken not to change during the EPSP, only the lower frequencies will be useful for detecting the resistance changes. Second, the height of the EPSP will not remain the same in the presence of the depolarization effected by the injected current since the depolarization resulting from an EPSP depends upon both the magnitude of the local conductance change and the existing transmembrane driving voltage. Thus, to the extent that the injected current produces a

local depolarization at the site of the EPSP generation, the depolarization produced by the EPSP conductance change will be reduced (Rall, 1967). Therefore, a diminution of the peak height of the voltage transient associated with a current injection occurring during an EPSP would be due to a combination of effects--one the effect of a reduced resistance on the voltage produced by a current source, and two, the effect of a reduced driving voltage on the current resulting from a transient conductance change.

The work of Smith, Wuerker and Frank (1967), published during the course of this investigation is addressed to the same problems that are considered here. The study of Smith et al, in cat motoneurons, involved a technique different from that used here. The present results are consistent with and complementary to the interpretation given by Smith et al of their results.

INTRODUCTION

Part II

The frog has a venerable place in the history of electrophysiology, but in intracellular studies it has largely been replaced by the cat; therefore, our knowledge, at the synaptic level, of motoneuron input-output relations and their role in producing motor function is drawn mainly from investigations with cat motoneurons. This situation tends to lead to thinking of the operation of vertebrate motor systems in terms of what is known about the cat. However, obvious as it may seem, a frog is not a cat, not even with regard to a basic motor system. Some of the differences in motor operation between frog and cat have been studied over the years (Bremer and Moldaver, 1934; Kuffler, Laporte and Ransmeier, 1947), but not with intracellular techniques applied to the intact frog.

Nearly all of the relatively few intracelluiar studies of motoneurons in anurans (frogs and toads) have used the excised spinal cord, and so it was not possible to examine the synaptic activity associated with different modalities or localities of peripheral stimulation, either electrical or "natural" (e.g. Machne, Fadiga and Brookhart, 1959; Araki, et al., 1953; Katz and Miledi, 1963). Only two previous studies involving intracellular recording in the spinal cord of intact anurans are known to this author (Kuno, 1957; Fukami, 1961), and both were done with toad. Only Fukami considered motor function; he considered just the effect of muscle sense on the motoneurons of the forelimb and gave the impression that the toad is a poorly developed cat. But the differences in motor function cannot be portrayed so simply as that.

This second portion of the thesis, using the intact preparation and

the recording techniques developed for Part I, elucidate, at the synaptic level in motoneurons, the interesting differences between frog and cat of the roles played by the muscle and cutaneous senses in determining motor activity. This study demonstrates that it is practicable to use intracellular techniques in investigating the frog motor system, a system that may be a prototype for those producing the more sophisticated functioning of purely land animals.

Anatomy

Studies on motor function in the frog must be concerned with the properties of the two types of motor systems that exist in this animal. Different investigators, depending upon their orientation, have applied various labels to these two motor systems. The preferred labels seem to be "small nerve" and "twitch" motorsystems (Kuffler and Vaughan Williams, 1953a,b), but other pairings such as "tonic" and "phasic" have been used. No pair is free from confusion because the same labels are applied with different meanings to types of motor systems in mammals. Referring to the frog, the twitch motor system has muscle fibers that exhibit a propagated action potential and a threshold; this is the type of muscle fiber that is found in mammals. The small nerve motor system of the frog has muscle fibers that exhibit only graded slow depolarizations that spread passively. The motor axons innervating these muscle fibers appear to fall into two populations defined by axon diameter and conduction velocity (Kobayashi, Oshima and Tasaki, 1952). The small nerve motor system includes axons of 2-8-μ diameter with conduction velocities of 2-8 M/sec, while the twitch motor system includes axons of 9-20-μ diameter with conduction velocities of 8-35 M/sec. The small nerve and twitch motor systems appear in most of the limb muscles, but their proportion varies from muscle to muscle.

One source of muscle afferents, the muscle spindles or stretch receptors, is innervated quite differently in frog than in mammals. In mammals the intrafusal muscle fibers are innervated by small diameter motor axons from a motoneuron population (the γ system) that is distinct from that supplying the extrafusal muscle fibers (Granit, 1955). In the frog, however, the motor axon that supplies extrafusal fibers also provides the motor control of the spindle through a branch to the intrafusal fibers (Katz, 1949, 1961). How such an arrangement of one motoneuron supplying both intrafusal and extrafusal muscle fibers is used in feedback control has not been explored.

Motor Activity for Posture

The studies of Kuffler and Vaughan Williams (1953a,b) have shown that the small nerve motor system has properties that suggest that it serves to maintain postures. This notion was further supported by the work of Chambers and Simcock (1960), who showed that the forelimb extensor rigidity in the decerebrate toad was due to overactivity of the small nerve motor system. The tonic nature of the small nerve system versus the phasic nature of the twitch system was also previously demonstrated by Bremer and Moldaver (1934).

Far more is known about the bases of postural activity in mammals than in frogs and toads. Of prime importance for the maintainance of postures in mammals are the reflex responses of motoneurons to the sensory inputs from muscle and tendon receptors--the proprioceptive reflexes (Sherrington, 1906; 1910). In frogs and toads, however, such reflex responses cannot generally be demonstrated (Mashima, 1955; Marx, 1950). Although the muscle afferents do not by themselves generally produce a discharge of frog motoneurons, the recordings of Moldaver (1936) and of Marx (1959a,b) indicate

that the muscle afferents do have a subthreshold excitatory effect on the motoneurons. Also, Mashima showed that proprioceptive inputs facilitate the skin reflexes. The case for the absence of a monosynaptic stretch reflex was given support more recently by the intracellular studies of Araki (1960) and Fadiga and Brookhart (1960) on excised toad and frog spinal cords. For both of these animals it was found that the monosynaptic EPSP associated with dorsal root stimulation did not result in motoneuron firing. This is in sharp contrast to the results of similar experiments with the cat where the monosynaptic dorsal root inputs are quite effective in producing motoneuron firing. If in the frog and toad the muscle sense by itself is so ineffective, how, then, can these amphibians utilize this information about the state of their limbs in establishing posture and controlling phasic motor activity? This thesis will show, at the synaptic level, that the cutaneous sense is the basic input required for the frog to perform motor functions.

Brondgeest's experiments with spinalized frogs in 1860 showed that posture and tone are reflexes maintained by sensory inputs from the periphery; but he did not investigate their origin--whether muscular or cutaneous. Later work on mammals, especially that of Sherrington, demonstrated the prime importance of muscle sense for mammals. However, later work on frogs suggested that for the most part the skin sense was dominant. Often these experiments (Ozorio de Almeida and Piéron, 1924a,b; Wertheimer, 1924) involved partial to complete flaying of the animal; such drastic modifications of the animals serve to make results from these experiments less than compelling. Use of various anaesthetics to suppress the skin sensibilities also resulted in a loss of ability to maintain posture. But here, again, there is a caveat, since the profuse circulation in the skin permits easy

access of the anaesthetic to the muscle sense. More recent experiments (Mashima, 1955) using electrophysiological techniques rather than gross observation support the conclusion that the posture reflex observed by Brondgeest was due mainly to sensory input from the skin rather than from the muscle.

Although the functions of the monosynaptic or stretch reflex is hardly restricted to simply enabling an animal to resist gravity, it is possible that in some sense an equivalency may exist between the strength of the monosynaptic reflex and the extent to which an animal exhibits antigravity postures. In mammals decerebrate and cerebellar rigidity is an over-activity of "antigravity" muscles, be they extensors or flexors. This rigidity was called by Sherrington "exaggerated posture." Now the posture and locomotor acts of frogs have little, if any, antigravity component in the hindlimbs, but the forelimbs do show some antigravity components. Toads show more antigravity activity than do frogs, especially with regard to the hindlimbs. Consistent with the above, decerebration or cerebellectomy in frogs and toads results in little or no change in the hindlimbs but does result, in toads, in rigid extension of the forelimgs (Abbie and Adey, 1950; Chambers and Simcock, 1960). In frogs the forelimb response after cerebellectomy appears not to be part of an exaggerated upright squat but, rather, an over-activity of the clasp reflex.

Phasic Motor Acts

The twitch motor system of the frog is probably primarily involved with locomotion and other phasic movements such as the various wiping reflexes. For this division of the motor system, there are several experiments that suggest that the monosynaptic reflex is not playing nearly as important a role in the behavior of anurans as it does for the behavior of mammals. If

the dorsal roots associated with a limb of a toad are cut, the animal continues to be able to use that limb in a well-coordinated manner in either walking or jumping or swimming (Weiss, 1936; Lissmann and Gray, 1940, 1946). This behavior is in striking contrast to that of mammals where de-afferentation of one limbrenders it unusable in locomotion. Even if all but one pair of roots are cut, the toad is still able to move with a slow, distorted, but still rhythmic quadrupedal gait. The degree of distortion in these movements seems to be related to the number of dorsal root fibers left intact rather than to the specific pair of dorsal roots not cut. Weiss claimed that the toad could perform rhythmic locomotion even if all of the dorsal roots were cut, but Lissmann and Gray found that one pair had to be left intact for the animal to show any semblance of walking. However, they did find that with all dorsal roots cut the toad still showed the alternate extension and flexion associated with labyrinthine stimulation. Also, the animal could still perform wiping and cowering movements and could sometimes swim.

Deafferentation in amphibians does not produce the disintegration of basic patterns of motor coordination to nearly the extent that it does in mammals. In amphibians, the operation of each limb appears to be much less tied to its own peripheral sensory receptors than is the case for mammals. The central connectivities of nervous elements serving motor function in the frog seems to be such that very generalized, or non-specific inputs can evoke a coordinated motor output. However, what appears to be a relative lack of dependence upon specific spatial sensory monitoring probably goes hand in hand with what seems to be the relatively limited locomotory repertoire of the frog. The second part of the thesis is an examination of the synaptic properties associated with muscle and cutaneous inputs to frog

motoneurons with the goal of obtaining clearer notions on how a frog is not a cat.

METHODS

Part I

Dissection

The experiments were performed on medium-sized (75-95 g) male frogs (Rana pipiens) in both summer and winter seasons. During the dissection and experiment, the animals were immobilized with d-tubocurarine (Squibb; approximately .002 cc/g initially); anaesthetics were generally not used. The majority of the experiments were done with animals with an intact central nervous system; in a few cases chronic spinal animals were used.

The basic dissection consisted of a dorsal laminectomy of vertebrae 5 through 9 followed by reflection of the underlying venous plexus. The venous drain from the spinal cord which feeds directly into the spinal vein on the plexus, was kept intact by careful tearing of the plexus with two fine forceps. The dura and arachnoid membranes were reflected in a similar manner resulting in the exposure of half of the spinal cord from the entrance region of dorsal root 8 to the filum terminale. To allow the microelectrode to penetrate into the spinal cord, the pia membrane was nicked in several places with a specially sharpened and shaped microknife. This technique was superior to using two forceps to tear the pia, and by using it with care the pia could be slit without any surface bleeding and with only slight penetration of the microknife into the cord. The dorsal and ventral roots were generally left intact. The circulation in the surface vessels of the cord was not interrupted and remained strong for three to four days if the animal was kept in a refrigerator (38° F) between experiments.

The ipsilateral leg was dissected to expose for stimulation the following nerves: R. profundus anterior, R. profundus posterior (branches

descendens communis and semimembranosus), and R. cutaneus dorsi pedis lateralis (Ecker and Wiedersheim, 1896). R. profundus anterior is a muscle nerve innervating extensors of the knee. R. profundus posterior is predominantly a muscle nerve innervating flexors, adductors and levators of the knee. This nerve does have a small cutaneous component innervating the middle of the inner surface of the thigh. R. cutaneous dorsi pedis lateralis is a cutaneous nerve innervating the skin of the outer side of the dorsum of the foot. These nerves were freed from the surrounding tissue but were usually not severed. In some cases the whole sciatic nerve in the thigh was freed, and in others the corresponding contralateral nerves were prepared.

Chronic spinal animals were prepared by removing the first two vertebrae and using a small suction probe to remove a cross-section portion of the spinal cord between the 2nd and 3rd roots. The suction technique left both the arterial and venous spinal circulation intact, and animals so prepared could be kept alive for more than six months.

The animals were placed in special apparatus which held them by a spring-clamp along the vertebrae and a support in the mouth. The movement of the spinal cord was reduced by allowing the trunk of the animal to hang free (Figs. 2 and 3). To prevent the frog from drying out those skin areas not involved in the experiment were covered with gauze pads kept moist by regular application of oxygenated tap water. The exposed spinal cord and peripheral nerves were covered with mineral oil. The experiments were carried out at room temperature to 21-24° C.

Stimulation

Excitation of each nerve was achieved by using two platinum, or two Ag/AgCl wires, to deliver 0.1 msec pulses that were isolated from ground. Isolation was provided by an isolation transformer, or by use of a special

stimulus isolation circuit (designed by Dr. K. Kornacker) that could be adjusted to confine stimulus spread. This second device was especially useful for this work, since stimulus spread can be a problem in the frog, because the various nerve bundles are of short length and close together. The stimulation pulses were obtained using standrad Tektronix equipment. In order to deliver two closely spaced pulses of different intensities to the same nerve, two Tektronix 161 Pulse Generators were connected to a common pair of stimulating electrodes but were isolated from each other by diodes at their outputs.

Recording

Microelectrode recording was done in the portion of the spinal cord between the 8th and 10th roots. The intracellular microelectrodes used were the standard glass capillary type and were usually filled with 2 molar potassium citrate by the vapor-pressure technique. Their resistance in a solution of potassium citrate (2 molar) was 30-40 MΩ; electrodes of less resistance did not usually result in stable penetrations. The preamplifier used was DC high input impedance (FET) unity gain device with negative capacity compensation (designed by Dr. J. Lettvin). The output of the preamplifier was displayed on one beam of a dual-beam oscilloscope (Tektronix 502), and the DC level was monitored on a second oscilloscope (Tektronix 503). In some of the experiments the signal from the microelectrode was recorded using an Ampex Tape Recorder so that computer (PDP-4) averaging of repetitive responses could be done.

The incoming volleys to the spinal cord were monitored by using a monopolar, Ag/AgCl electrode on the 9th dorsal root. The signal from this electrode was AC amplified and then displayed on the second beam of the Tektronix 502. The animal was grounded through an Ag/AgCl plate clipped to the back muscles.

Of the numerous motoneurons penetrated, only those having a resting potential of 60 mV or more were considered as representative for this study. Such a motoneuron could be held on average for about 20 minutes.

Mathematical Model

To obtain an indication of the feasibility of detecting a dendritic impedance change, a model similar to that of Rall was analyzed. The model is more limited than Rall's but is sufficient to give insight into the difficulties of this study. In the model the dendrites are represented as an infinite coaxial transmission line in parallel with a lumped representation of the cell body as a resistance and capacitance. The model was designed to simulate the effect of dendritic membrane resistance changes on the voltage response to somatically injected current. The development of the model and the relevant equations are shown in the Appendix.

Figure 2. Overview of the experimental apparatus showing the frog in the holder and the positioning of its limbs. See Fig. 3 regarding the locations of the recording and stimulating electrodes.

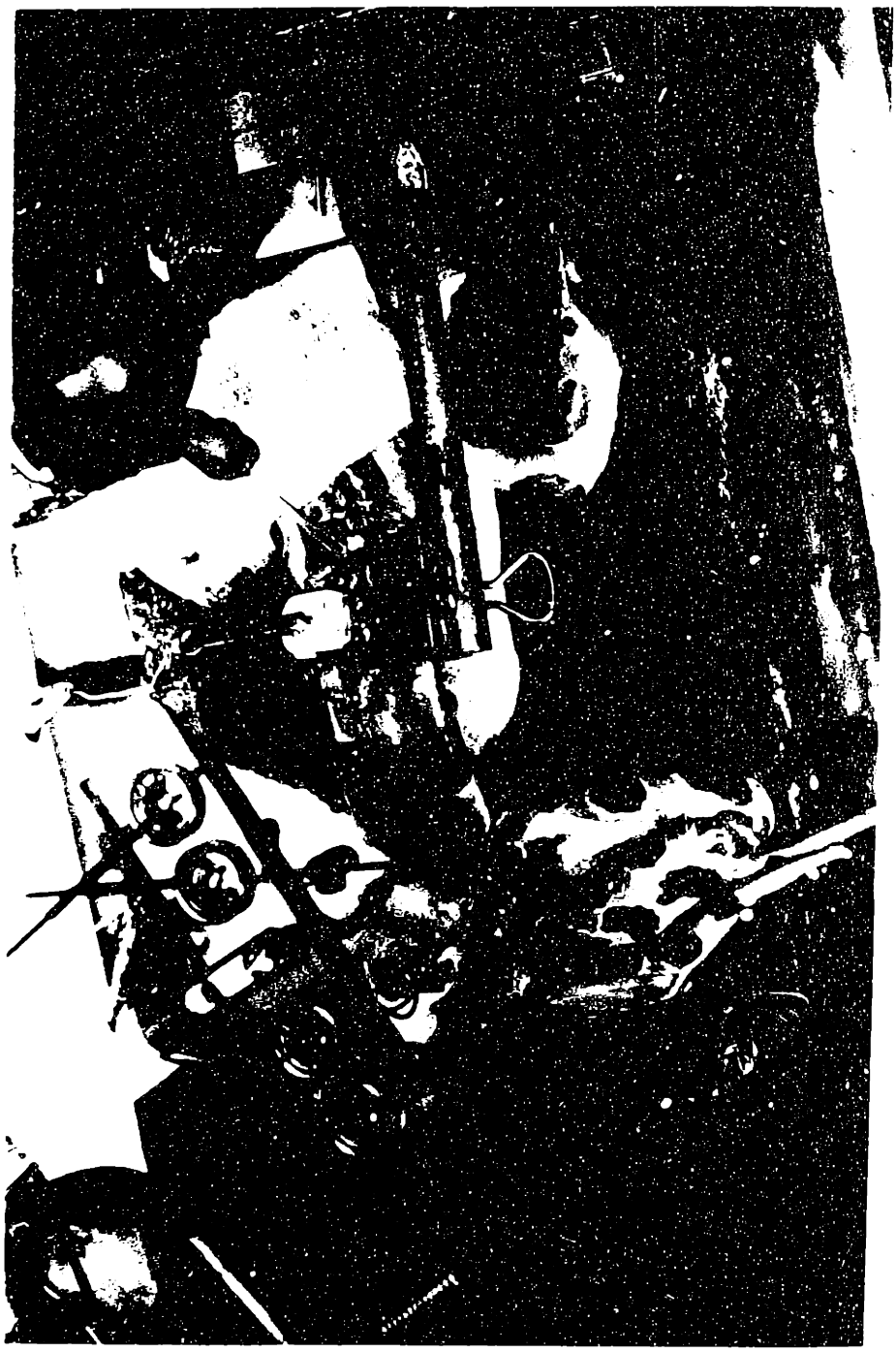


Figure 3 . A frog in the holding apparatus with the stimulating and recording electrodes in position. The three electrodes on the left are the stimulating electrodes on the peripheral nerves. The recording electrode on the 9th dorsal root and the microelectrode are in the center.

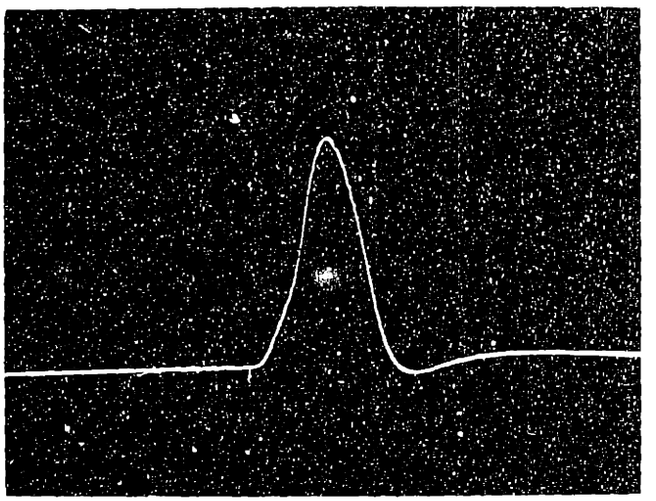
Part II

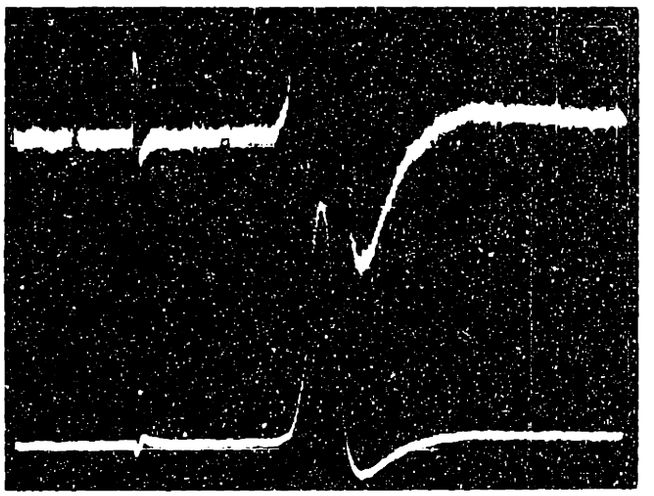
The methods used in this portion of the study were much the same as previously described but with the addition of a technique providing "natural" stimulation of the skin. The "natural" stimulation was generally restricted to punctate touch and pressure applied manually using a double-ended probe attached to the end of a very flexible cantilever wand. One end of the probe was a slightly dulled No. 26 hypodermic needle, and the other end was a 1/8"-diameter ball of epoxy. The output from a strain-gage bridge mounted on the cantilever served as a qualitative monitor of the stimulus intensity and as a trigger signal for the recording apparatus.

Figure 4a . Intracellular record of typical full antidromic spike in motoneuron. The A-B, or IS-SD inflection point is clearly visible on the rising phase. Spike height 74 mV. Resting potential 70 mV. Time marks on trace .5 msec.

Figure 4b . Anaother example of the full antidromic spike, illustrating the most common form of the after-potential. The difference between cat and anuran in the form of the after-potential has been previously noted by Araki et al. (1953). The conduction velocity of the motor axon was about 17 M/sec. Top trace 5 mV/large div; bottom trace 20 mV/large div. Time scale 1 msec/large div.

Figure. 4c . Intracellular record of motoneuron response to stimulation of peripheral muscle nerve, indicating effect of membrane potential on after-potential. The after potential is somewhat corrupted by the presence of an EPSP, but the tendency for the after-hyperpolarization to become less with increased membrane potential is clear. The change in membrane potential occurred as the result of slight movement of the microelectrode. Conduction velocity about 22 M/sec. Top trace 1 mV/div; bottom trace, DC, 20 mV/div. Time scale 2 ms/div.





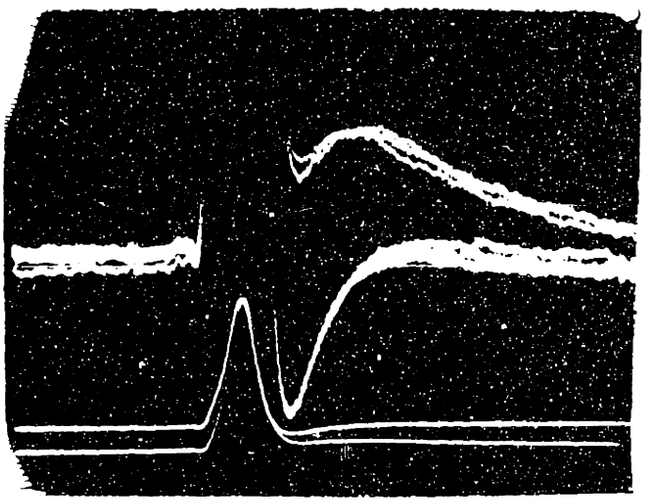
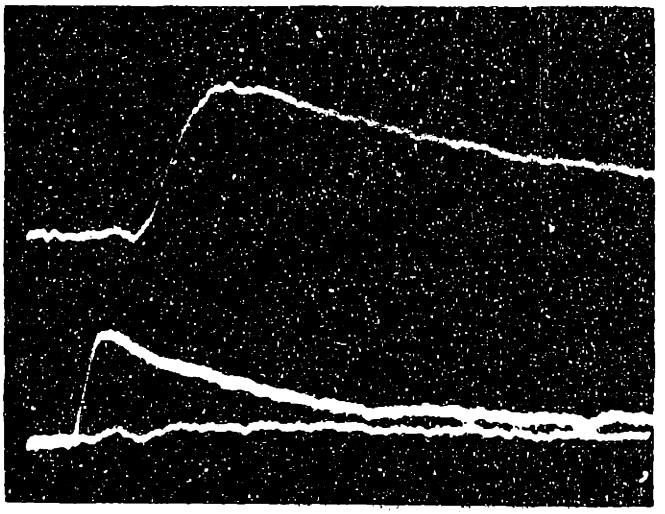
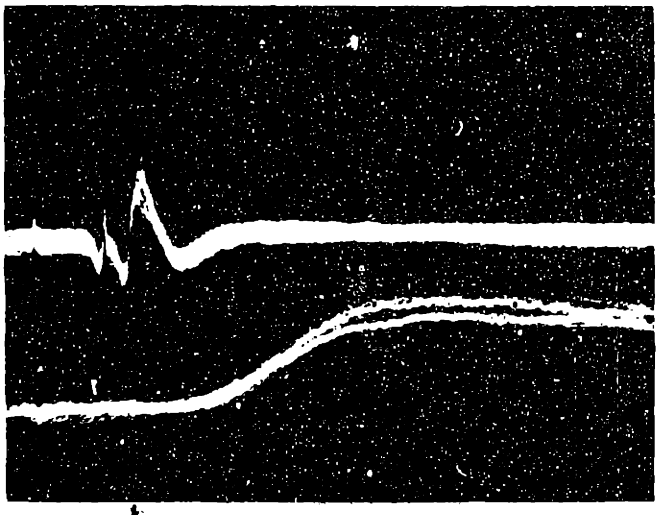


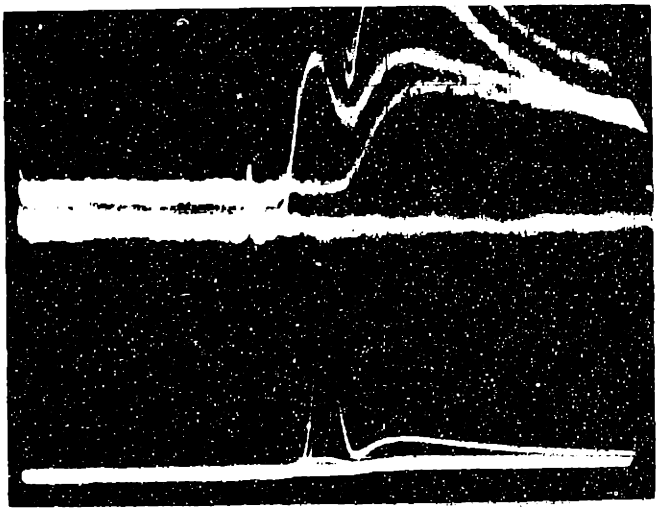
Figure 5a . Monosynaptic EPSP produced by maximal stimulation to peripheral muscle nerve at 60/min. Top and bottom traces are intracellular and extracellular response respectively, time scale 2 ms/div. Middle trace is at 5 ms/div with stimulus intensity reduced. All traces at 2 mV/div.

Figure 5b . Intracellular response to stimulation of peripheral nerve at an intensity just subliminal for actuation of the motor axon. One antidromic spike occurred (see fiducial mark for origin) permitting an indication of the synaptic delay. Time scale for both traces, 1 ms/div. Bottom trace 1 mV/div. Top trace at 200 μV/div is the compound action potential recorded by a monopolar electrode on the 9th dorsal root. Polarity for the dorsal root recording is negative up. Polarity for the intracellular record is positive up.

Figure 5c . Intracellular recording. Peripheral muscle nerve stimulation showing the various combinations of antidromic and orthodromic responses. Top traces and bottom traces show the same responses but at different gains. Top traces are AC at 2 mV/div; bottom traces are DC at 20 mV/div. Time scale is 2 msec/div. The full antidromic response is shown to block partially resulting in the M spike, and to block completely resulting in only the orthodromic response--the monosynaptic EPSP. The height of the full antidromic spike is about 80 mV.





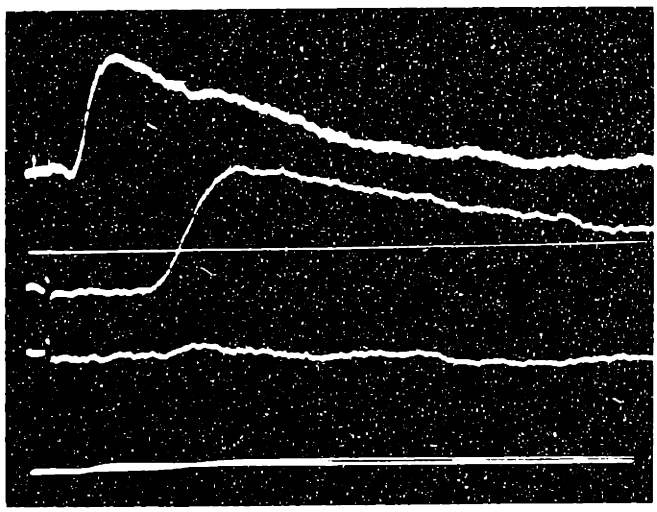


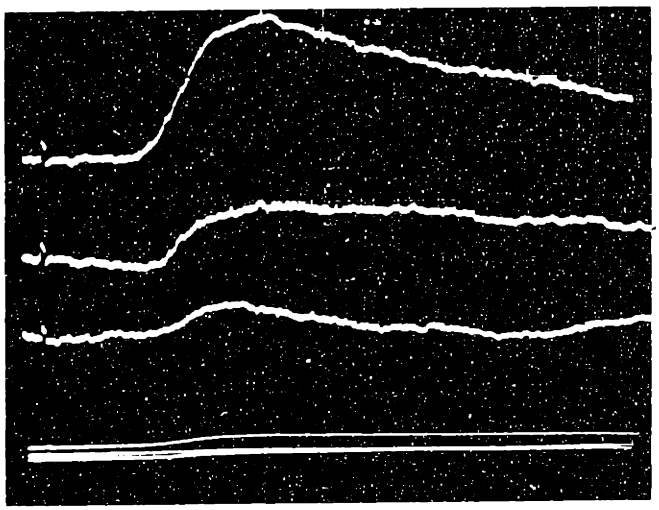
in a summation great enough to discharge the motoneuron. This finding is consistent with the work of Fadiga and Brookhart (1962). But this result is markedly different from the situation in the cat where, with stimulation of a muscle nerve, the homonymous monosynaptic EPSP usually can reach the threshold for discharge and result in the monosynaptic reflex. In addition, while in the cat the monosynaptic EPSP from muscle afferents shows post-tetanic potentiation, this was not found for the corresponding monosynaptic EPSP in the frog.

In order to study the monosynaptic input from dorsal root stimulation in the isolated spinal cord, Fadiga and Brookhart (1960, 1962) had to suppress the internuncials with sodium pentobarbital to eliminate the polysynaptic responses. Curiously enough, when using the intact frog and stimulating at 60/min, the suppression is done by the nervous system itself. That is, with maximal stimulation for myelenated muscle afferents resulting in a multi-grouped input as monitored on the 9th dorsal root, only a monosynaptic EPSP is seen in over 90% of the motoneurons, whether "flexors" or extensors. As the stimulus intensity was increased from threshold, the monosynaptic EPSP simply increased in size while maintaining its "simple" shape (Fig. 6, 6). No inhibitory effect from possible tendon afferents were seen at 2-3 times threshold for group I. Although the frog is reported not to have the group II spindle afferents fround in cats, it does have muscle afferents other than spindle group Ia (Ito, Toyama and Ito, 1964); these other afferents produced no effect at a frequency of stimulation that is considered slow for the cat. However, if the frequency of stimulation is decreased by an order of magnitude to 6/min, or if the animal is spinalized, the situation becomes quite different. These changes will be described in Part II where they are more relevant. It is sufficient for Part I to indicate that only

Figure 6a . Additional examples of monosynaptic EPSP's. Maximal stimulation R. prof. post., branch descendens communes, at 60/min 10th dorsal root and 9th and 10th ventral roots cut. Two traces DC at 20 mV/div to show resting potential. Three other traces at 1 mV/div. Top trace at 5 ms/div. Middle trace sane stimulus as top bit at 2 ms/div. Bottom trace at 2 ms/div is the extracellular response.

Figure 6b . The same cell as above but showing the effect of varying the stimulus intensity. Intensity decrease from top down. Top three traces at 1 mV/div with a time scale of 2 ms/div.





monosynaptic dendritic EPSP's were produced in the intact frog by stimulating the muscle afferents at 60/min.

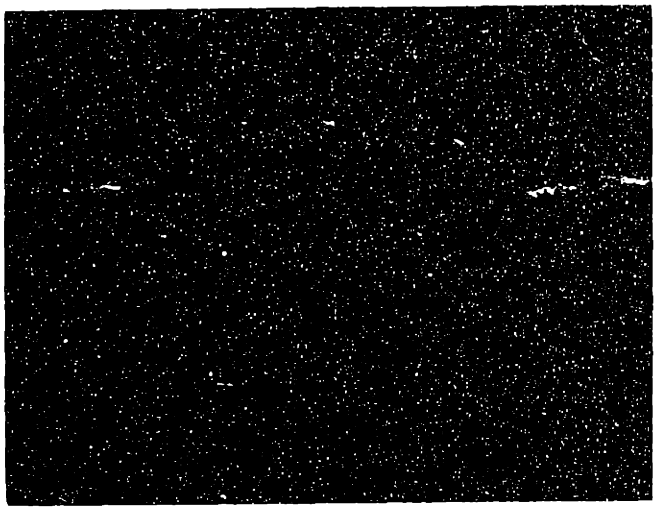
Stimulating the peripheral muscle nerve to antagonist muscles regularly produces inhibitory PSP's in the cat. But such stimulation at 60/min in the frog only rarely resulted in hyperpolarizing PSP's (Fig. 7). In those cases where no PSP was produced by "antagonist" stimulation, there was no effect on the synergistic EPSP when the two stimulations were presented at various times relative to each other. Thus, an inhibitory shunting effect without an associated hyperpolarization appears not to be the case. The absence of IPSP's at a stimulation rate of 60/min is probably another manifestation of the general blocking of multi-synaptic pathways which, as described above, resulted in the presence of only monosynaptic EPSP's from homonymous stimulation. Also, it should be pointed out, that other investigators, using the excised cord, found few IPSP's until special methods were used to allow their detection (Kubota and Brookhart, 1963a).

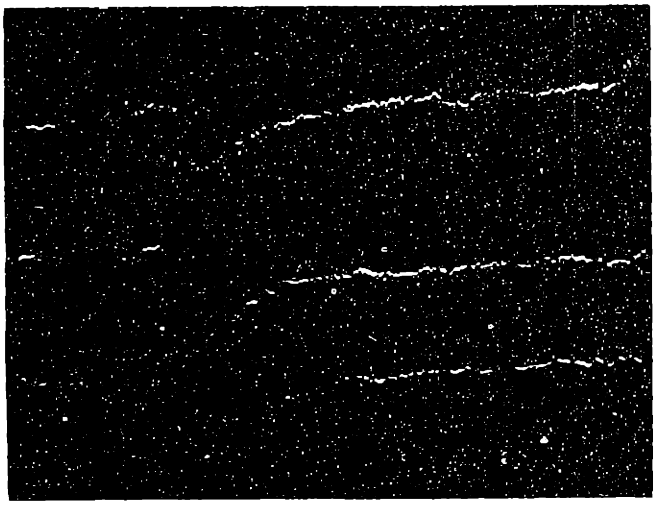
In about half the cases, stimulation of the muscle nerve of "antagonists" produced monosynaptic EPSP's that had the same time course as EPSP's produced by stimulation of the muscle nerve in which the motoneuron had its axon (Fig. 12). In this regard no striking difference between extensor and "flexor" mononeurons was seen. The appearance of these EPSP's is very likely the result of the fact that the muscles served by R. prof. ant. are not truly antagonists of the muscle served by R. prof. post.

Antidromic Spike Interactions

While gaining familiarity with the preparation and the intracellular techniques, some interaction experiments were done using the EPSP complex produced by stimulation of a cut dorsal root and the full antidromic spike resulting from stimulation of a peripheral muscle nerve. Although this

Figure 7 . Examples of hyperpolarizing synaptic activity rarely seen in response to stimulation at 60/min. Top records, stimulation of muscle nerve at 60/min. Bottom records, stimulation of cut 9th dorsal root. All records at 2.5 ms/div and 1 mV/div.

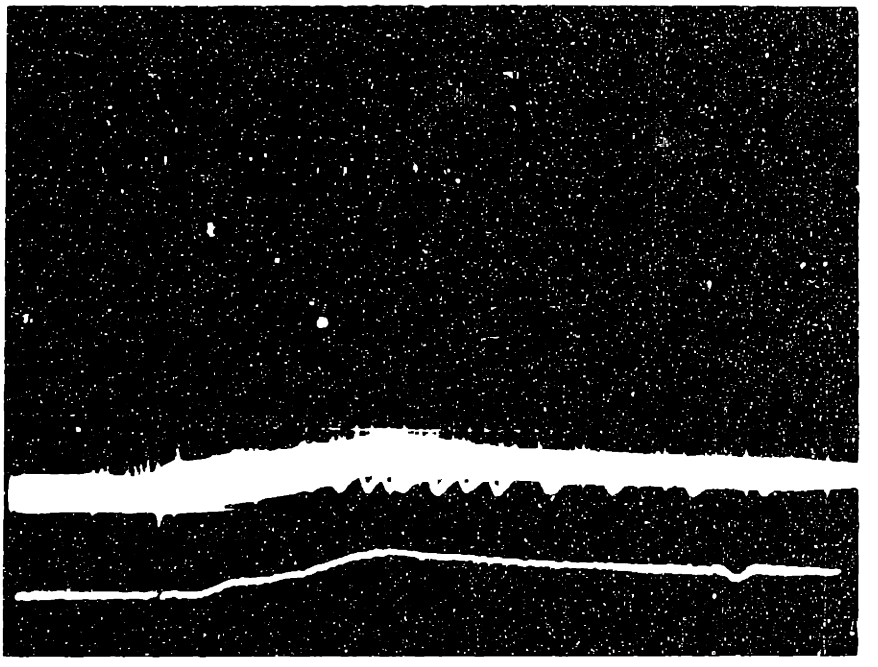




approach to trying to indicate a shunting action of synapses in motoneurons has been cautioned against (Coombs, Eccles and Fatt, 1955b), Machne, Fadiga and Brookhart (1959) and Fadiga and Brookhart (1960) found that a reduction of about 5% in the height of the antidromic occurred frequently but only during the presence of the polysynaptic EPSP's and not during the monosynaptic dendritic EPSP. In the present experiments the data is generally in agreement with that of Brookhart, but the interpretation is different (Fig. 8, 6, 9). It was observed that the contour of the spike peak in time was suspiciously similar to the time course of the extracellular potential associated with the EPSP complex. Now, in the frog, as contrasted to the cat, there is a strong tendency for failure of invasion of the antidromic spike to occur with regard to both the axon hillock and the soma (Grinnell, 1966; Brookhart, and Kubota, 1963); the chance of invasion is greatly enhanced by the presence of EPSP's. The occurrence of this enhanced invasion of neighboring motoneurons is reflected in the local extracellular transient voltage as a marked increase in size--up to 2 mV--of the negative-going spike associated with antidromic invasion (see Fig. 15, 6). Thus, in order to begin to interpret relatively small height changes of intracellular events, one must know the corresponding extracellular transients, since they are the bias on which the intracellular transients are riding. Brookhart's group does not present records of the associated extracellular transient voltage, so one cannot say to what degree his observation reflects synaptic membrane resistance changes (shunting) as opposed to changes in the extracellular transients. It is believed that the changes observed in this study in the height of the intracellularly recorded antidromic spike during the EPSP complex basically reflect extracellular events rather than synaptic shunting. This caveat adds to previous cautionary statements regarding the use of the antidromic spike

Figure 8a. Interaction of the full antidromic spike with the PSP complex resulting from stimulation of the cut 9th dorsal root. Lower trace is the intracellular record for the orthodromic response alone. Note that although the spike height goes to a lower point during the later portion of the PSP complex, it goes to a slightly higher point during the earliest portion. This pattern is consistent with the shape of the extracellular transient voltage associated with the orthodromic response. The control height of the antidromic spike alone is given by the first three responses on the left. Further explanation in the text. All responses at 20 mV/div and 5 ms/div.

Figure 8b. Example in another motoneuron of the interaction described above. In this example the A-B break is seen to occur at the same point during the course of the PSP complex. Further explanation in the text. All responses at 20 mV/div and 2 ms/div.



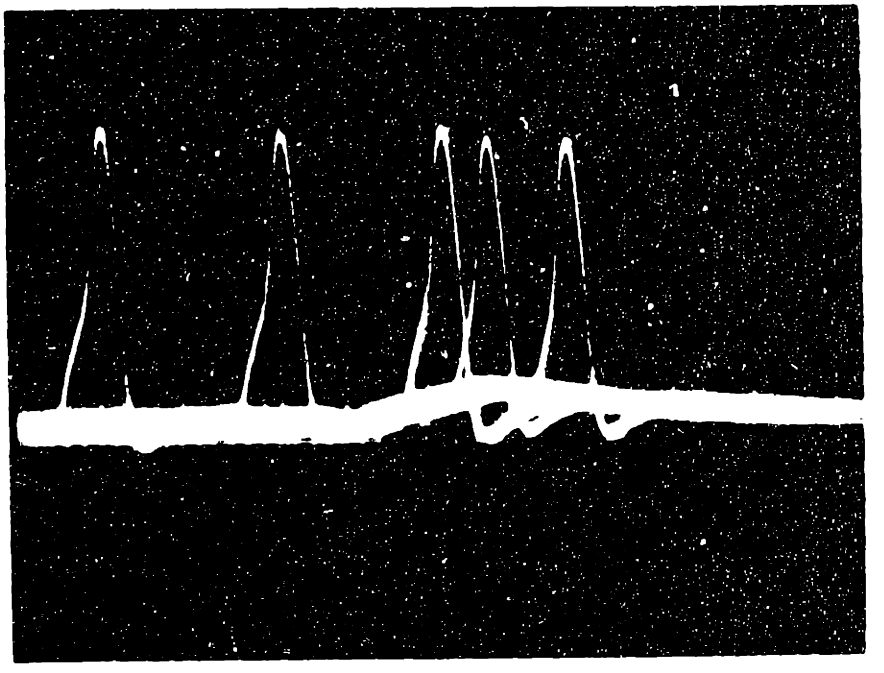
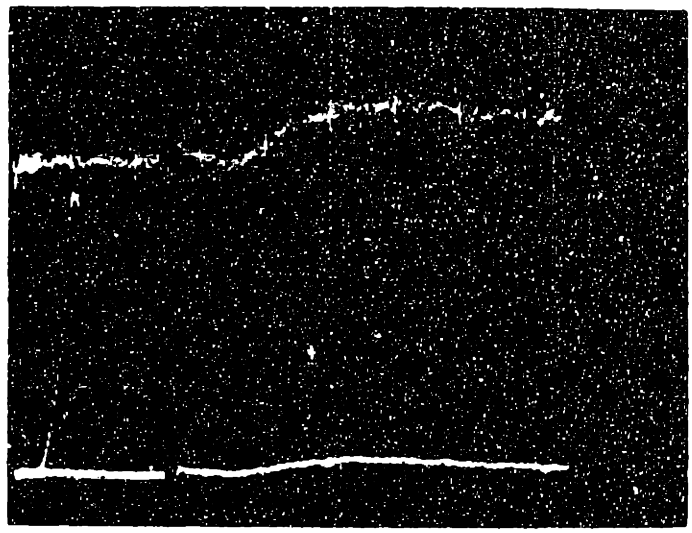


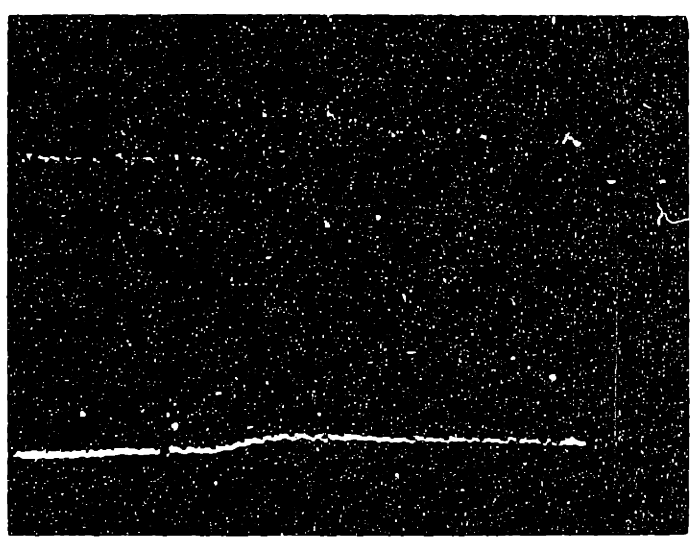
Figure 9 . Examples of an alternate display technique for illustrating the interaction between the antidromic spike and the EPSP resulting from stimulating the muscle nerve not containing the motor axon of the motoneuron. By applying a train of brightening pulses to the Z axis of the oscilloscope, a selected portion of the interaction response was obtained. The pulse train was of fixed duration and frequency as the antidromic spike was "marched through" the orthodromic response. The technique made evident slope changes in the antidromic response. The pulse train began at the movement of stimulation of the antidromic axon in the periphery. Thus, the contour of the orthodromic response (the base line) is comprised of a series of the latency intervals. In the three records the upper trace is at 5 mV/div and the lower trace is at 20 mV/div. The time scale is 2 ms/div.

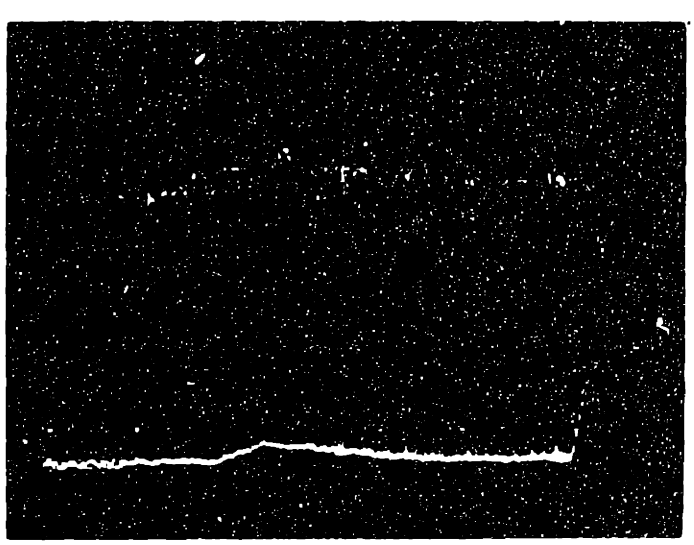
In the top record the duration of the brightening pulse train extends to the peak of the antidromic spike. The drop in the spike height persists too long to represent exclusively the brief membrane resistance transient of the EPSP. See text for the effect of enhanced invasion in neighboring motoneurons on the height of the antidromic responses.

The second record is from the same motoneuron as above but with a reduced stimulus intensity for the EPSP response. The duration of the brightening pulse train was decreased. The frequency was decreased also. The A-B break appears to remain at a fixed point. The increase in slope of the soma invasion response is seen as an increase in the height reached by the brightening pulses.

The third record is from another motoneuron. The pulse train duration is not long enough to display the peak of the antidromic response when it occurs alone. Again, the A-B break appears to remain at a fixed point.







height to indicate changes in neuronal properties; it can be used (Kuffler and Eyzaquirre, 1955), but confounding factors must be controlled, or be fortuitously absent if an unequivocal result is to be obtained.

Types of Antidromic Responses

One of the problems associated with trying to use the full antidromic spike as an indicator of the membrane resistance change associated with synaptic activity is that the invasion of the cell body is of itself a transient resistance change that is much greater than that associated with the usual synaptic activity. Thus, a microelectrode with its assumed location in the cell body sees the interaction of two processes involving resistance changes, but since it is electrically as near to, or nearer, the larger resistance change process (the invasion), it can indicate little if any interaction. This problem becomes more severe the farther away is the locus of synaptic activity from the recording microelectrode.

A microelectrode situated in the motoneuron cell body reveals that there are three types of antidromic responses associated with the stimulation of a peripheral motor axon. One type (Fig. 4a-c) is the full antidromic spike with an inflection point on the rising phase (the A-B spike of Fuortes, Frank and Becker, 1957; the IS-SD spike of Coombs, Curtis and Eccles, 1957b). This response is believed to reflect the existence of the action potential processes over the soma and perhaps part of the proximal dendrites. The second type is a spike that goes to only about the inflection point (the A spike of Fuortes et al., 1957; the IS spike of Coombs, et al., 1957b). This response is believed to indicate blockage of the action potential at the axon-soma junction and, thus, no active discharge of the soma. The third type of response, the M spike, is a small (2-4 mV) depolarization that is believed to reflect blockage of the action potential near where

the insulating myelin sheath stops at the distal end of the initial axon segment (Fig. 5c). Thus, the A and M spikes, which are due to the passive, or "electrotonic," spread of current, reflect the failure of the action potential to propagate beyond one of two regions of lowered "safety factor."

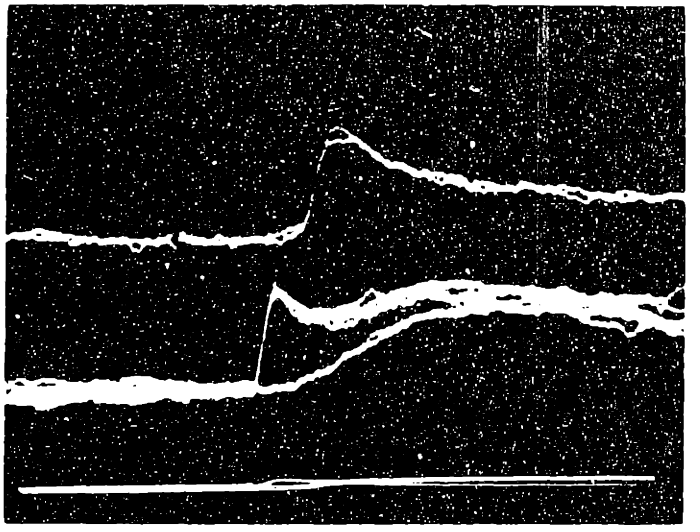
By using the A or M spike in interaction studies, one might expect to alleviate the problem described above, since now the resistance change associated with the test signal would be occurring farther away from the recording electrode. Put into other words, what is desired is a current source for the test signal, since then the voltage produced by it would be a qualitative measure of the cell impedance. However, even with an ideal current source for a test signal, the changes of voltage associated with pairing it with an EPSP is not simply a reflection of a membrane resistance change related to the EPSP. There is a second factor which enters, because the test signal produces electrotonic changes in the membrane potential throughout the cell, and, thus, alters the driving potential for the local EPSP process. Therefore, the EPSP does not remain of constant size but becomes smaller or larger depending upon whether the test signal depolarizes or hyperpolarizes the membrane. Rall (1967) has suggested, on theoretical grounds, a method of separating the contribution of the two effects by comparing the PSP response in the presence of depolarizing and hyperpolarizing currents, but because of the nonlinear membrane resistance during hyperpolarization, it does not seem experimentally promising. The appearance of an "apparent shunting" of a depolarizing test signal would be consistent with the impedance transient model of the synaptic mechanism, but it does not exclude other models which involve processes dependent upon membrane voltage.

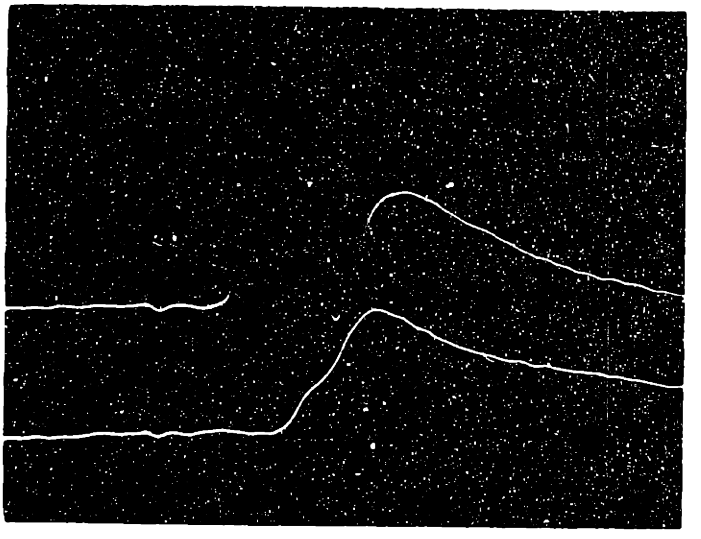
Figure 10. Examples of some of the types of intracellular activity seen in motoneurons in response to stimulation (60/min) of a peripheral muscle nerve.

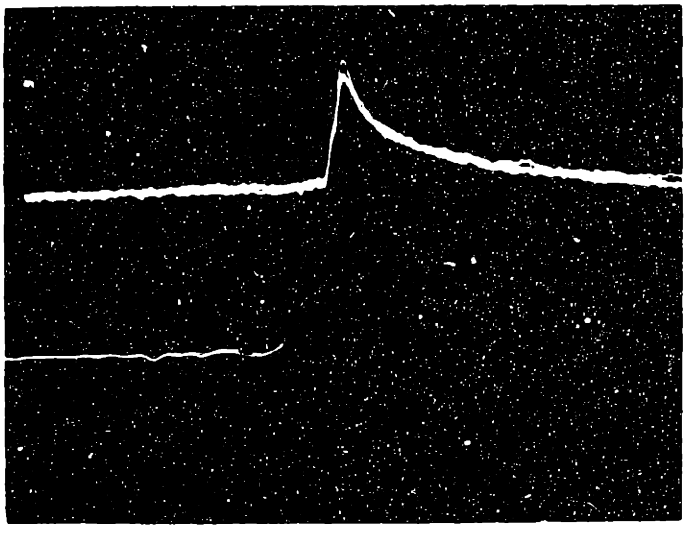
The top set of records are from a motoneuron of R. prof. ant. All traces at 1 mV/div. and 2.5 ms/div. The lower traces are responses to stimulation of R. prof. ant. and at two stimulus intensities. At the lower intensity only the monosynaptic EPSP is obtained while at a slightly higher intensity the antidromic M-spike response is also obtained, preceding the EPSP. The upper traces illustrate a depolarizing response to stimulation of R. prof. post., branch descendens communis; because of the relatively fast rise time of this response it is believed to be a ventral root EPSP rather than the much more usual orthodromic EPSP. Responses which had the shape characteristics of ventral root EPSP's (see Kubota and Brookhart, 1963b) were seen only infrequently; this observation is consistent with the finding of Grinnell (1966) that ventral root EPSP's usually disappeared at temperatures above 18°C.

The middle and bottom sets of records are from a motoneuron of R. prof. post. and illustrate responses to stimulation (60/min) of R. prof. post., branch descendens communis. In the middle set of records (1 mV/div and 1 ms/div) the lower trace shows a depolarization with an inflection point on the rising phase. Such a form of response was seen only infrequently. On the basis of latency times it is believed that such a response represents the combination of a monosynaptic orthodromic EPSP and a ventral root EPSP rather than the combination of a monosynaptic and a disynaptic EPSP. The upper trace illustrates the occurrence of the antidromic spike following a slight increase in the stimulus intensity.

The bottom set of records illustrates several repetitions of the response seen in the lower trace of the middle set of records. All traces are at 1 mV/div. Upper traces are at 5 ms/div; lower traces are at 1 ms/div.







A-Spike Interactions

The A-spike response occurred "spontaneously" in some motoneurons, and in others the full A-B response gave way to only the A-spike response for a minute or more after high frequency antidromic stimulation. Interaction of the A spike with the EPSP complex associated with dorsal root stimulation most often resulted in the peak of the A spike going to the same value of membrane voltage no matter what region of the EPSP complex it was combined with (Fig. 8,9). Such compensatory adjustments between the height of the A spike and the size of EPSP suggests that, in fact, the peak of the A spike reflects more the existence of a voltage source as viewed by the microelectrode than the hoped-for approximation to a current source. That is, for the A spike, even though the active impedance changes are farther removed from the recording electrode than in the case of the full antidromic spike, they are still close enough to dominant when in combination with synaptic resistance changes.

Interaction of the A spike with EPSP's results in changes in the A-spike parameters of latency and of time to peak, which indicate that the A spike as a test signal is corrupted to some extent. That is, the presence of the EPSP modifies the generation of the test signal in addition to presenting a new membrane state for the test signal to interact with. The presence of the EPSP results in a decrease in latency of the onset of the A spike and in an increase in the average rate of rise from onset to peak. If the distance, and thus the resistance, between the location of the block in the initial segment and the cell body is considered to vary with the degree of depolarization produced by EPSP's, then some of the changes associated with the interaction begin to become understandable. Therefore, a detailed analysis is not needed to conclude that the A spike

is too imperfect a current source to be reliable in indicating resistance changes of the relatively small magnitude associated with most EPSP's.

The M-Spike as a Current Source

The shortcomings of using the full antidromic spike or the A spike as a test signal pointed the way to consideration of the M spike. Although both the full antidromic spike and the A spike have been used in interaction studies reported in the literature, the author is not aware of any previous attempts to use the M spike. One of the probable reasons for this is that the great majority of intracellular studies done on motoneurons has been done with cats, and with cats the M spike is not frequently seen by itself (Coombs et al., 1957b). However, as mentioned previously, in the case of frog motoneurons, failure of complete antidromic invasion is occasioned by the appearance of only the M spike as frequently as it is by the appearance of the A spike, and failure of invasion in some form is much more common in "healthy" frog motoneurons than in "healthy" cat neurons.

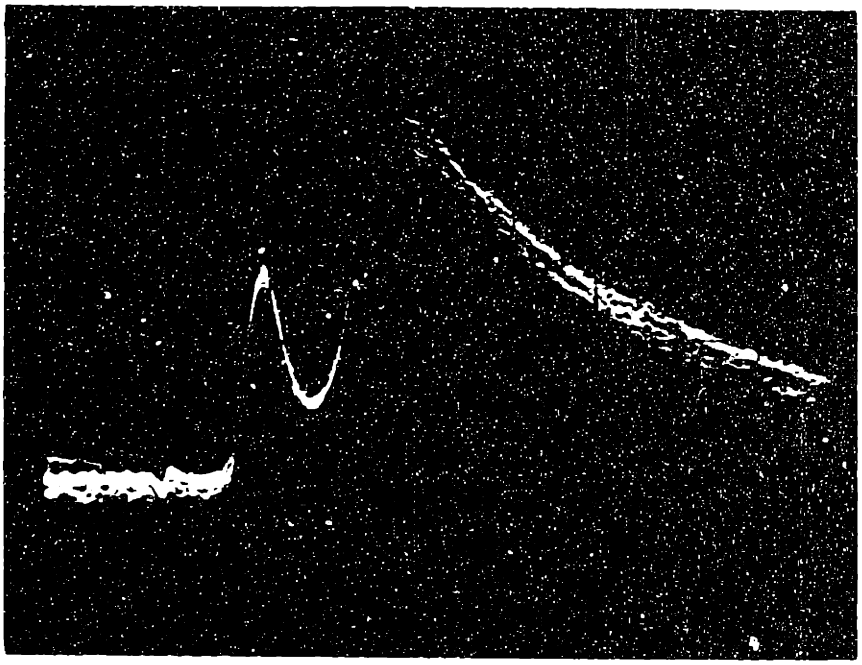
Condiseration of the relative geometrical location of the origin of the M spike indicates that it is a much better approximation to the desired current-source test signal than is the A spike. The M spike is the manifestation in the soma of an antidromic action potential blocked about 50-100 μ from the soma. The axonal core resistance between the block region and the soma is calculated to be about 10-15 MΩ, while the whole neuron resistance as seen by the microelectrode is 1-2 MΩ. Although these DC figures do not necessarily reflect the behavior of what in fact is a transient signal in a distributed system, they do probably indicate that the M spike is an improvement over the A spike with regard to being a current source. Another way of stating the improvement afforded by using the M spike as the naturally occurring test signal is to indicate that the membrane resistance changes

involved with the generation of the test signal are now further removed from the recording site and that excitatory depolarizations have a much reduced effect on the parameters of the test signal. Depolarization of the cell by either microelectrode-injected current, or by synaptic activity, does produce a slight decrease in latency to peak but much less than in the case of the A spike. In short, the M spike is the natural test signal that is most nearly a current source with regard to the soma-dendrite impedance and the available position of the recording electrode in the cell body.

M-Spike Interactions

The use of the M spike as a test signal was confined to interaction with only the monosynaptic EPSP's associated with muscle afferents, since the thesis search was for an indicator of the resistance change associated with dendritic synapses. Experiments were done in over 50 motoneurons that had stable M spikes for several minutes or more. Both the EPSP and the M spike were produced by stimulation of peripheral muscle nerves at 60/min so as to produce only monosynaptic dendritic EPSP response (see above). Since the stimulation producing the M spike was done on a mixed (afferents and efferents) muscle nerve, the response seen in the motoneuron was the M spike followed by the monosynaptic EPSP produced by the afferent fibers that were activated along with the efferents (Fig. //). The EPSP usually had a lower threshold than the M spike, but the size of the EPSP could be increased by increasing the stimulus intensity beyond that required to produce the M spike. In the early experiments with the M spike, the monosynaptic EPSP to be examined was produced by stimulation of the second peripheral muscle nerve (Fig. 12). This procedure resulted in a depolarizing PSP in about half the motoneurons and in no voltage changes in the other half. The absence of "antagonist" IPSP's, apparent, masked or inverted, has been

Figure // . Example of the intracellular response in a motoneuron to stimulation (60/min) of a muscle nerve when the antidromic response is the M spike. The first event is the M spike and the second event is a monosynaptic EPSP resulting from stimulation of the afferents in the peripheral muscle nerve. Twenty-five superimposed sweeps at 2 ms/div and 1 mV/div, AC recorded. The fluctuations in the base line and in the size of the EPSP, which confound the M-spike technique, are clearly evident. Computer averaging of ten of the responses is shown in the lower record.



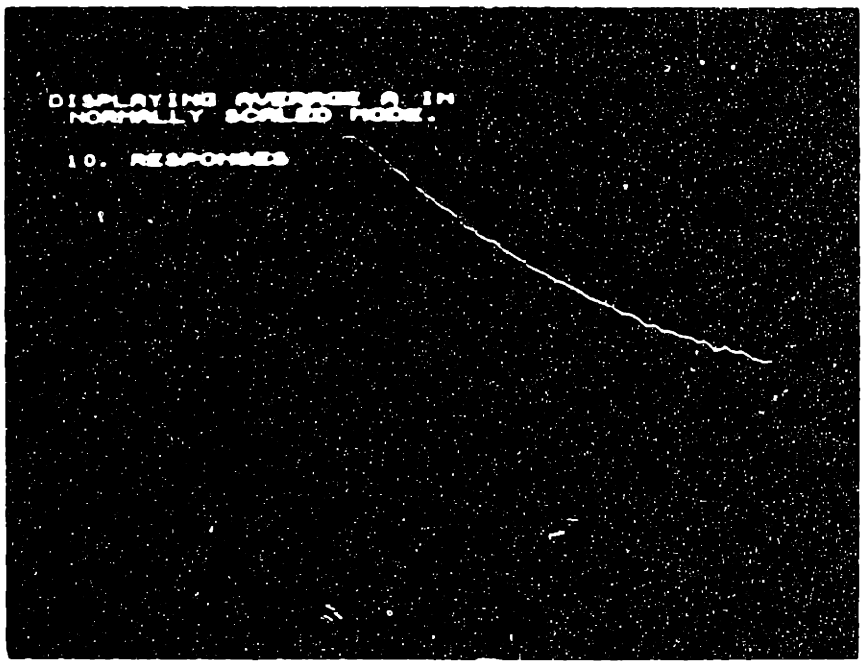
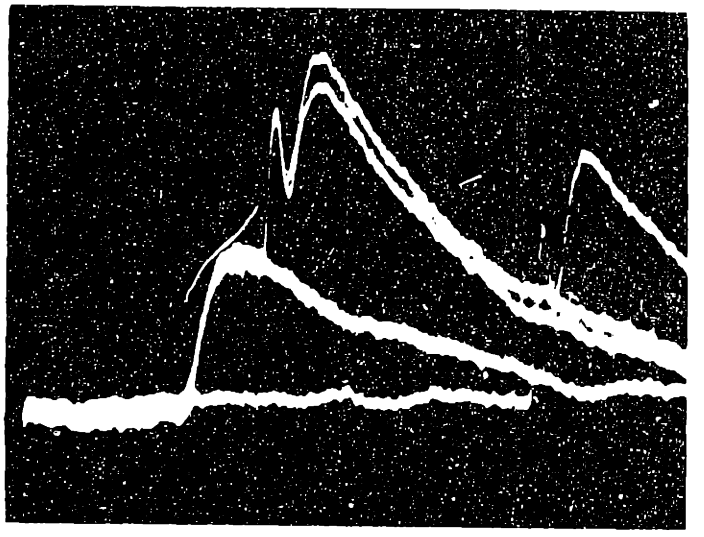

DISPLAYING
NORMALLY SCALED MODE.
10.

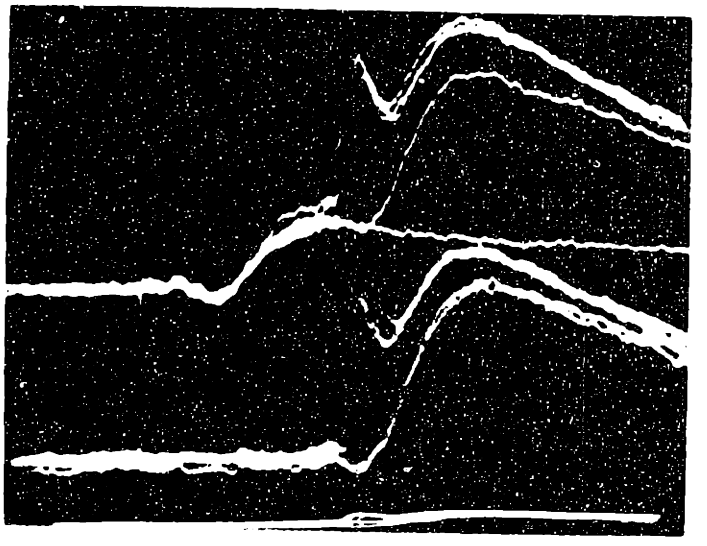
Figure 12 . Three sets of intracellular records in three different motoneurons illustrating the interaction of the M spike with the monosynaptic EPSP produced by stimulating the muscle nerve not containing the motoneuron axon. The top and middle records are among those most favorable for showing a diminution of the M spike. On the basis of the intracellular records alone a reduction in M-spike peak height of 4-5% was sometimes seen. But experience with the technique has shown that factors such as the fluctuations in base line and in EPSP size make observations based upon superposition of one or two sweeps unreliable. When the extracellular transient voltage is considered, as it must be, then the diminution is found generally to be illusory (see text). All records at 1 mV/div. Top and bottom records at 5 ms/div; middle record at 2 ms/div.

In the top record the M spike and associated EPSP are seen alone on the right (see Fig. //). This response is interacted with the EPSP from the alternate muscle nerve. The interaction combination and the EPSP alone are seen in the center of the record. The heights of the two M spikes can thus be compared.

In the middle record the lower traces show the M spike response. The M spike does not occur, sometimes leaving only an EPSP response. In the upper traces the M spike is interacted with the second EPSP. Here, also, in one sweep the M spike does not occur.

In the third record a similar set of interactions are shown.





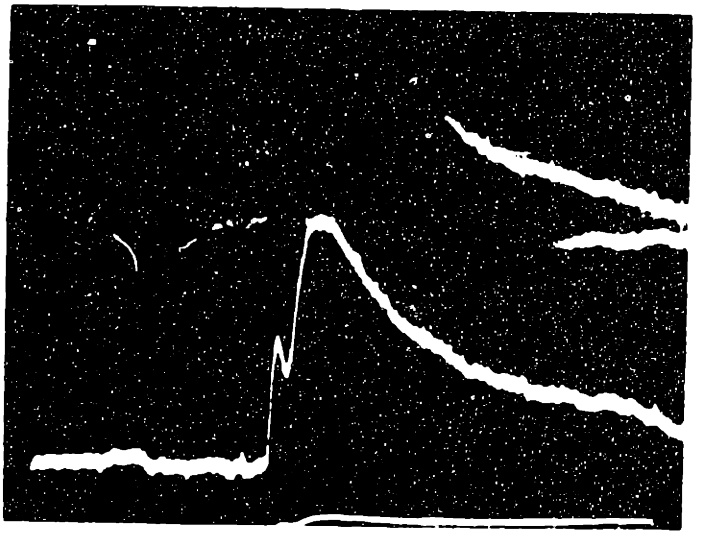
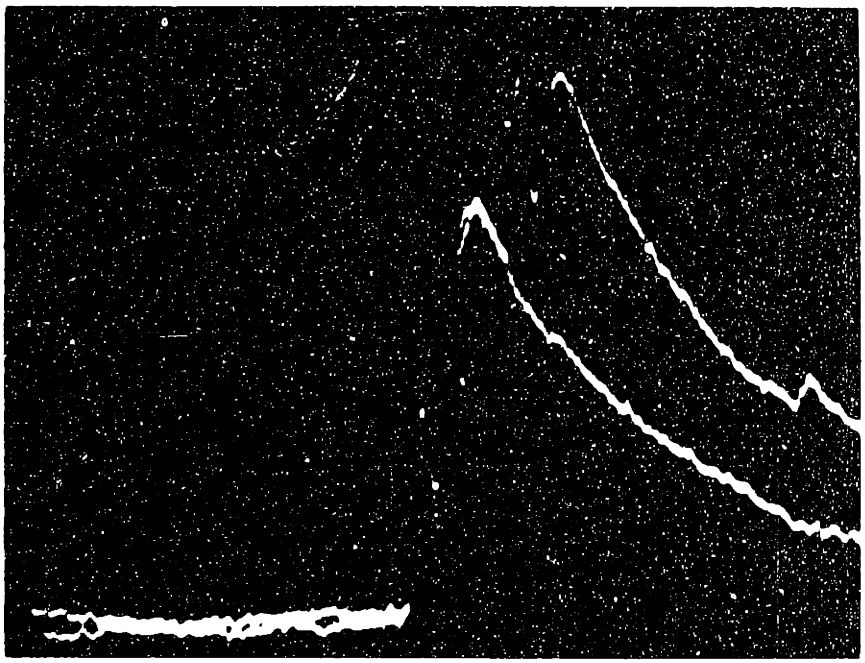
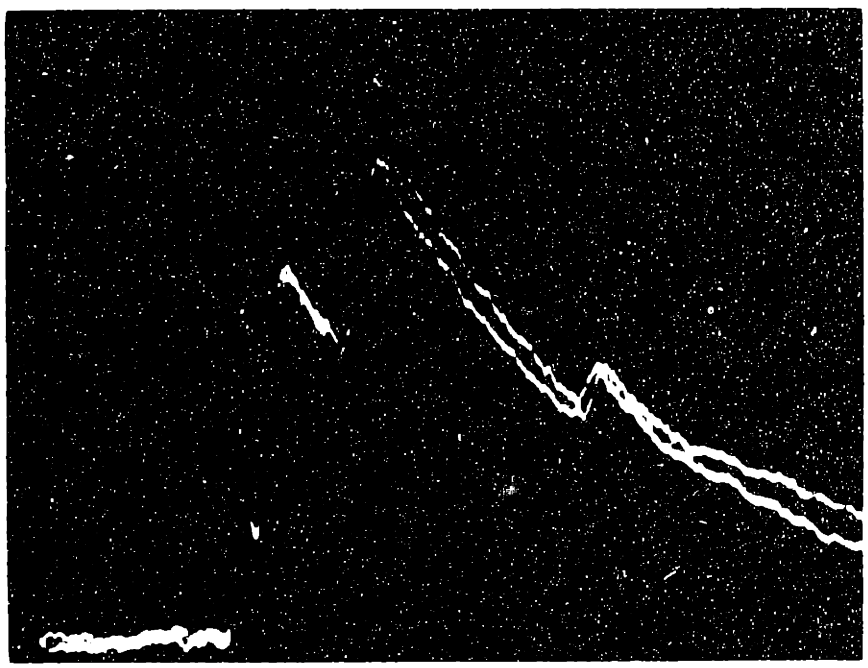


Figure 13 . Interaction of the M spike with the monosynaptic EPSP coincidently produced with it by stimulation of a peripheral muscle nerve. The second, interacting M spike is the result of a second stimulus to the muscle nerve. No diminution of the M spike occurs in the upper record. In the lower record when consideration is taken of the actual bias voltage on which the M spike is riding, as seen when the M spike does not occur, no diminution is found. Note that the EPSP resulting from the second stimulus to the peripheral nerve is much reduced in size. All records at 1 mV/div and 5 ms/div.





discussed above. In the later experiments advantage was taken of the difference in threshold between the EPSP and the M spike produced by stimulation of one muscle nerve. Two diode-isolated stimulators were used to produce a pair of stimulating pulses of different intensities; the first pulse, of lower intensity than the second, resulted in only the EPSP, while the second resulted in both the M spike and the EPSP. This technique allowed data to be taken on nearly all motoneurons with a stable M spike, for it removed the dependency on the existance of an EPSP from "antagonist" muscle nerve stimulation.

Initially, data was taken photographically from the face of the oscilloscope. A complete set of pictures included the three response patterns--M spike alone, EPSP alone, and M spike combined with EPSP--with first the microelectrode intracellular and then extracellular. In the earlier experiments the peak of the M spike was positioned at various points along the time course of the EPSP, but in the later experiments it was located just before the peak of the EPSP. In most cases measurement from the photographs revealed no change in the height of the M spike when it was combined with the dendritic EPSP (Fig. 13). In those cases where the intracellular records indicated a small (5% or less) change in M-spike height, the necessary consideration of the associated extracellular records revealed that the change was illusory (Figs. 12; 15a,b).

One of the problems in trying to detect small changes in the M-spike height was that in many cases the EPSP alone often itself changed size by about 5% from one stimulation to another. The M spike alone did not usually suffer this fluctuation. The data-taking procedure was also hindered by small base line changes. In later experiments an attempt was made to circumvent these problems by using a PDP-4 computer to average 30-50 taped

repetitions of each of the response types. This manipulation of the data did not bring out a consistent diminution of the M spike when it was combined with the EPSP. The usual finding was that the M spike remained the same (Fig.14b). In a few cases there was a very slight (about 3%) decrease of the M spike (Fig.14c), but such an occasional small change is hardly compelling indication of a dendritic resistance change acting as a shunt.

Mathematical Model of the Interaction

To obtain a better estimate of the degree of the shunting to be expected, a mathematical model of the neuron was constructed. The model borrows some from the approach used by Rall for his neuron models (1959a, 1964), but it is simpler and not as flexible since what was wanted here was simply a reasonable assessment of the difficulty involved in detecting transient dendritic resistance changes. The model is developed in the Appendix; it depicts the neuron as a parallel resistance and capacitance representing the soma in parallel with the complex impedance of a semi-finite coaxial transmission line representing the dendrites (Fig.3). This model indicates the response, as seen in the soma, to a current step coinciding with a uniform dendritic and/or somatic membrane resistance step. It also can represent the rising phase of an "EPSP" resulting from a uniform dendritic and/or somatic membrane resistance step. The difference between the current step response with and without a membrane resistance step reflects only the effect of the changed membrane resistance, since the concomitant effect on the EPSP driving potential is mathematically excluded by setting the membrane voltage and the EPSP reversal voltage both equal to zero.

Manipulations with this model convincingly demonstrate the extreme unlikelihood of detecting a distal dendritic membrane resistance transient

Figure 14a . Computer-averaged responses for M-spike interaction. Double stimulation to a single muscle nerve but with two different intensities results in antidromic M spike only for the second stimulation. Upper records are intracellular responses. Lower records are extracellular responses to, from top down: EPSP stimulus alone; M-spike stimulus alone; both stimuli combined. Consideration of both intracellular and extracellular responses indicates that no definite reduction in the peak height of M spike occurred.

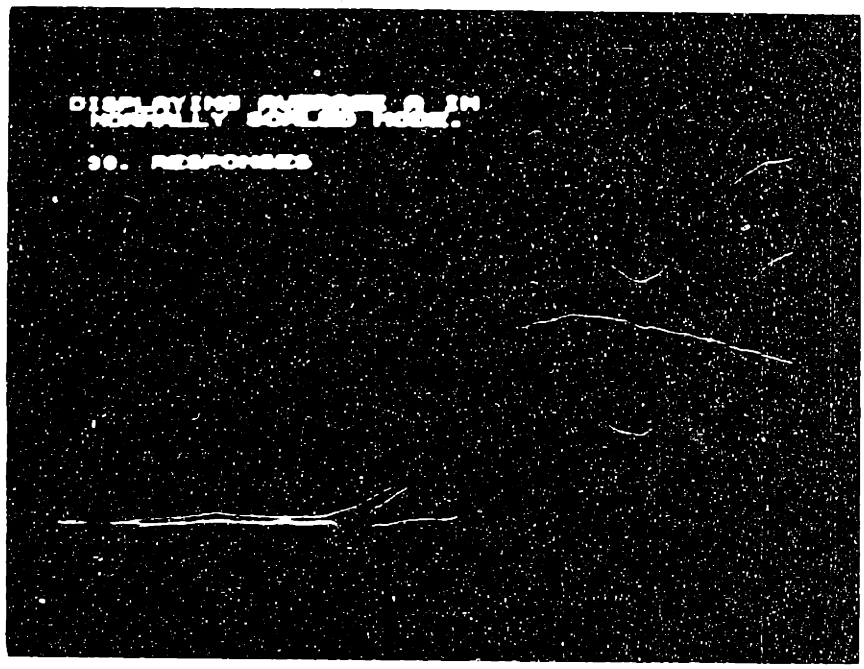
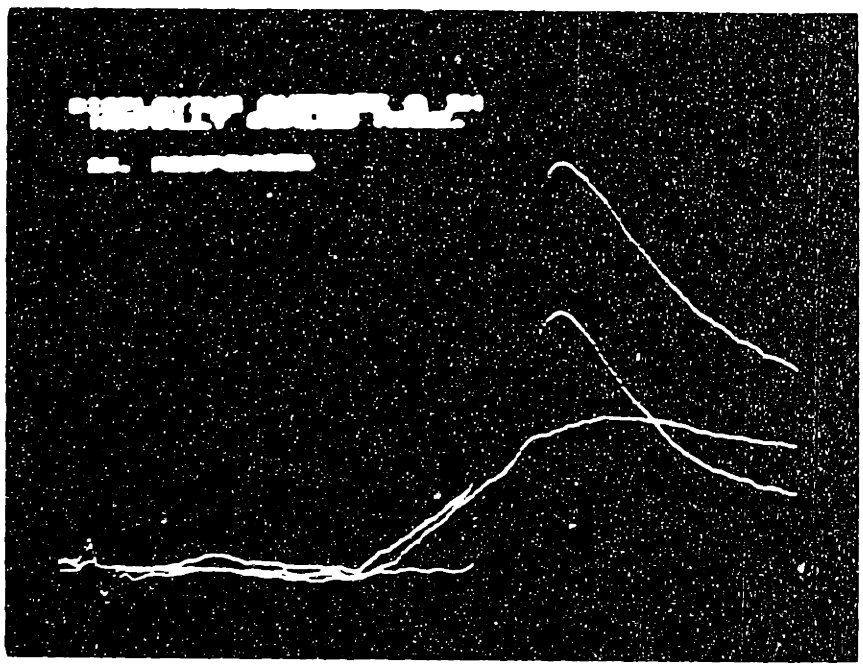


Figure 146 . Computer-averaged responses for M-spike interaction EPSP resulting from stimulation of the muscle nerve not containing the axon of the motoneuron. Upper records are intracellular responses. Lower records are extracellular responses to, from top down: M-spike stimulus alone; EPSP stimulus alone; both stimuli combined. Consideration of both intracellular and extracellular responses indicates that no reduction in the peak height of the M spike occurred.



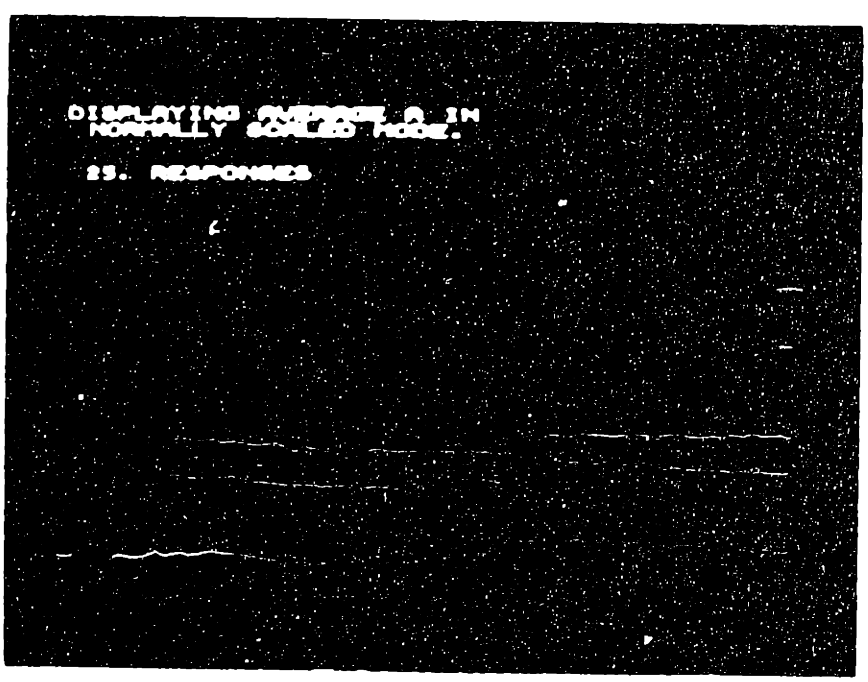
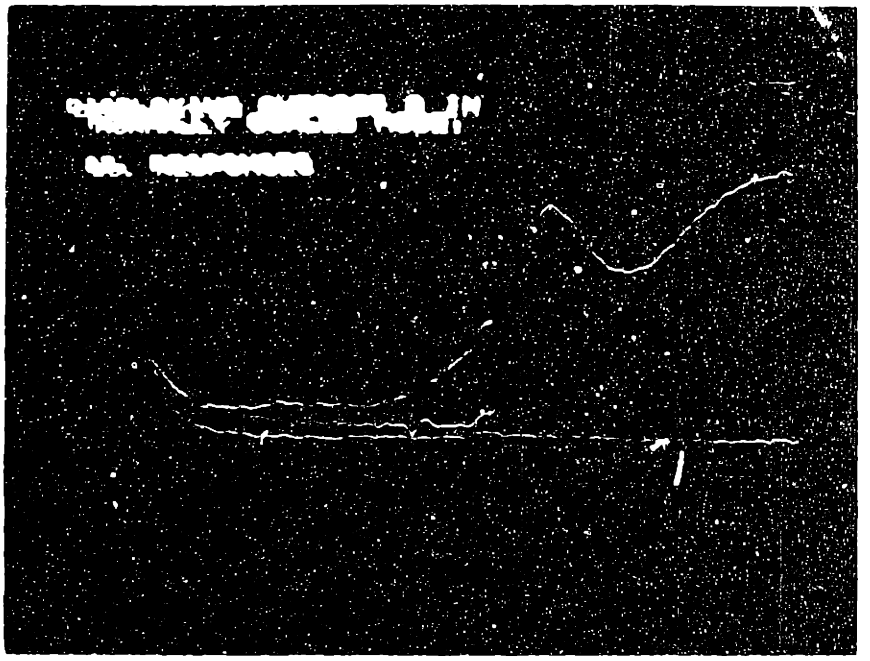

29. RESPONSES

Figure 14c . Computer-averaged responses for M-spike interaction. Double stimulation to a single muscle nerve but with two different intensities results in antidromic M-spike only for the second stimulation. Upper records are intracellular responses. Lower records are extracellular response to, from top down: Both stimuli combined; M-spike stimulus alone; EPSP stimulus alone; no stimuli. The calibration signal on the left of all records is approximately 1 mV, 1 ms. Consideration of both intracellular and extracellular responses indicate that a reduction (about 5%) in the peak height of the M spike occurred. This reduction is the largest seen with computer averaging.



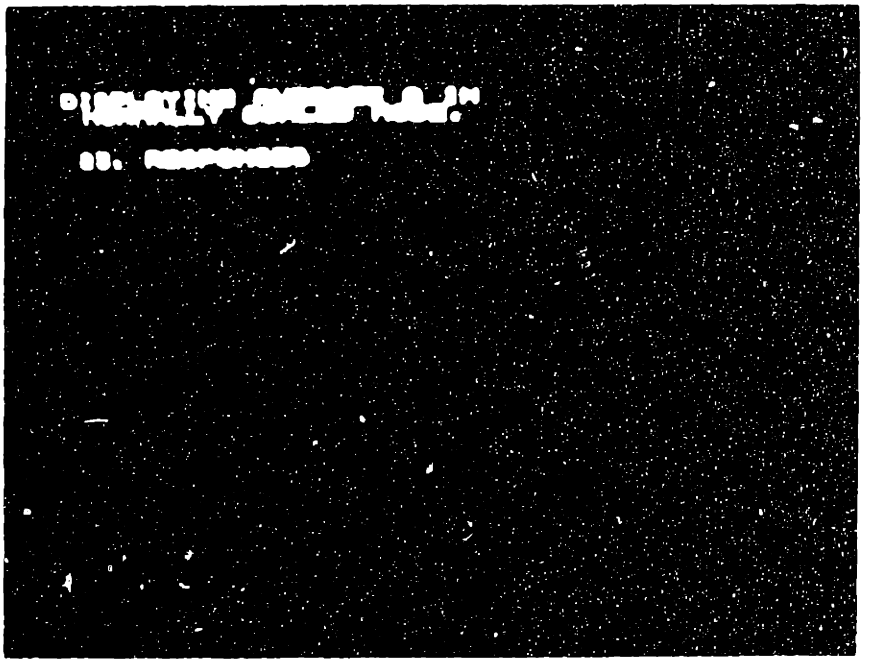
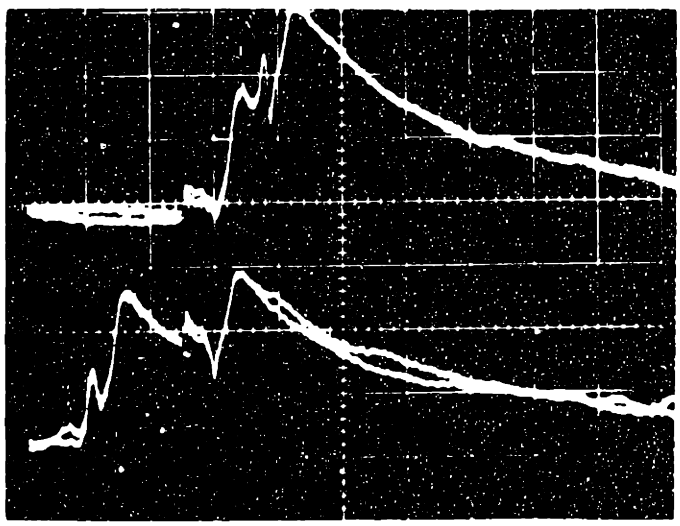


Figure 15a . Computer-averaged responses for M-spike interactions illustrating "illusory" diminution of M spike. Double stimulation of peripheral muscle nerve (see Fig. 13). Intracellular responses in upper record. Extracellular responses in lower record correspond to, from top down: Double stimulation; first stimulation alone; second stimulus when it follows the first stimulus (explanation in text). The intracellular records alone show a diminution of the second M spike when combined with the EPSP, but consideration of the extracellular response reveals that this diminution is illusory.

Figure 15b . Effect of enhanced antidromic invasion of neighboring motoneurons on the extracellular transient voltage. See Fig. 15a above. In upper traces M spike follows EPSP, and in lower traces M spike precedes EPSP. Note change in response just preceding EPSP. 2 mV/div and 5 ms/div.



when both the recording electrode and the testing current source have somatic locations. The model gives an upper bound to the degree of shunting existent in the experimental situation, since the model represents a resistance change over the entire dendritic surface, while for the frog motoneuron only the more remote portions of the dendrites are involved in the synaptic activity.

A set of results from the model will serve to point up the formidability of the task. For a dendritic resistance change in the model which produces a depolarization roughly equivalent to the larger EPSP's seen in the experiments, the decrease in the voltage transient associated with a current step is of the order of 1%, at times corresponding to the time of the peak of the M spike. In terms of the experiment this 1% figure translates to a signal of 20-30 μV in at least 200 μV of nerve! These figures are likely to represent those cases which are the more favorable for detection. In the case where an equivalent-sized "EPSP" is due to only somatic resistance transients, the figure for detectability increases by a factor of about 2.5 More detailed onsiderations of the predictions from the model are presented in the Appendix.

Part II

In Part I the preparation with the frog motoneuron was considered essentially as a system which provided the opportunity to investigate the properties of synaptic inputs that have a relatively defined dendritic origin; no particular reference was taken of how the motoneuron reflected the frog's motor behavior, which does indeed present some curious differences in comparison to that of mammals. Since the preparation is an intact one, it provides the opportunity to examine intracellularly the response of motoneurons to different classes of peripheral inputs. This type of study has not been previously done on the frog since the majority of intracellular studies on the frog motoneuron have used the excised spinal cord. The results of Part II combine with other more gross work, both electrophysiological and "cut-and-watch," to give a more detailed representation of amphibian motor function.

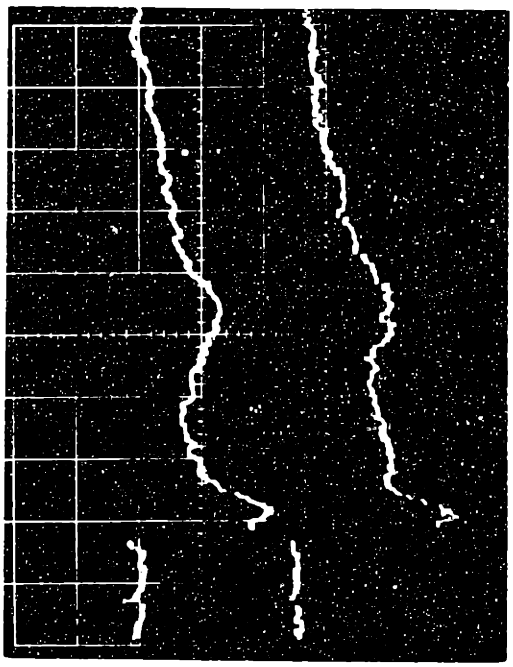
The population of motoneurons from which the results of this section were obtained included a number of the cells used in Part I as well as others that had a resting membrane voltage of at least 50 mV. The majority of the frogs were intact and immobilized to different degrees with curare; a few frogs were chronically spinalized and results with them will be specifically noted.

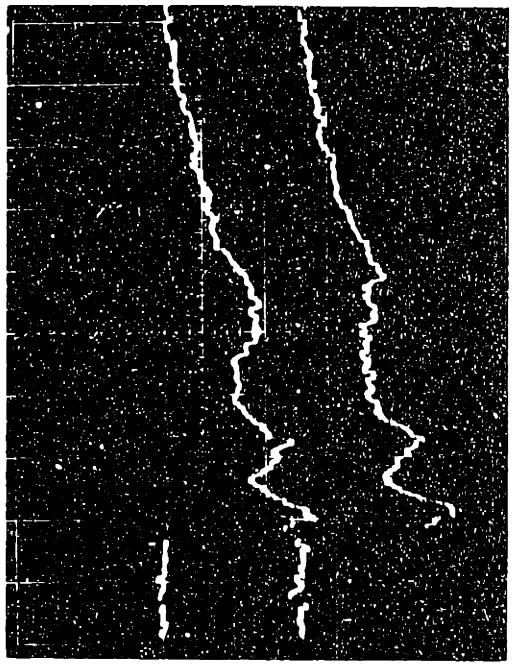
Frequency Effect on Synaptic Activity

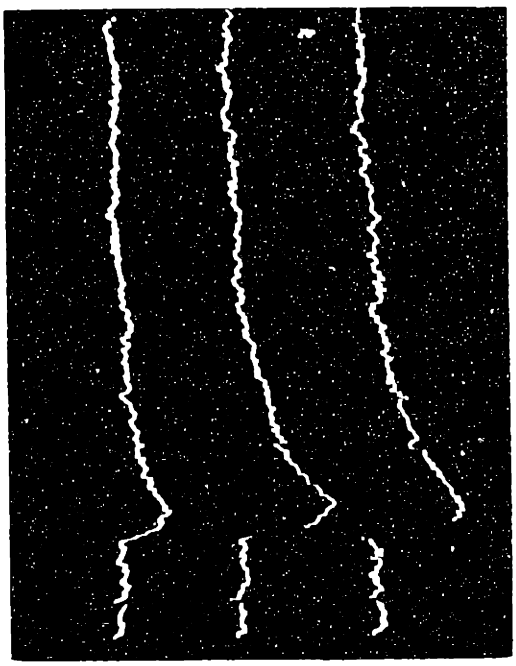
In Part I it was found that even supramaximal stimulation of the muscle nerves at a frequency of 60/min resulted in general in only the monosynaptic dendritic EPSP's in motoneurons and that these EPSP's could not be combined with themselves in any fashion (such as with PTP) to result in the firing of the motoneuron. Even in those instances when an entire 9th or 10th

dorsal root, or the sciatic, was supramaximally stimulated (for myelinated fibers) at 60/min, motoneuron discharge was quite infrequent, although polysynaptic activity was present. It was supposed that this polysynaptic activity was due to cutaneous inputs and, thus, the electrical stimulation of a cutaneous peripheral nerve was examined (R. cutan. dorsi pedis lat. serving the dorsal surface of the foot). The effect at a frequency of 60/min was quite dramatic through its absence. That is, in most cells there was no PSP activity even though the monitor electrode on the 9th dorsal root indicated a compound action potential, and the extracellular focal potential in the dorsal horn indicated synaptic activity in, presumably, the first order interneurons. Now, the extracellular spinal cord recordings of Bravo and Fernandez de Molina (1961) in the decerebrate and spinalized frog had shown the appearance of a delayed focal potential when the frequency of stimulation of a cutaneous nerve was less than about 10/min. Their observations suggested that at these low frequencies higher-order interneurons located in the intermediate gray matter became active. With the present preparation the basic findings of Bravo and Fernandez de Molina were confirmed (Figs. 16, 17). When the frequency of cutaneous stimulation was slowed from 60/min to 6/min, the motoneuronal synaptic activity went from essentially nothing to a PSP complex lasting tens of milliseconds. This dramatic contrast is illustrated in Fig. 18, 4 The input to the cord as reflected by the complex action potential in the dorsal root did not change with frequency (Fig. 19, 6). The effect of frequency on the production of this extended synaptic activity was graded in that the overall size of the complex activity decreased more or less continuously as the frequency was increased from 6/min to about 20/min, at which rate the PSP activity had usually disappeared. Some exceptions to this observation existed in the persistence

Figure 16 . Focal potentials in the dorsal horn in response to electrical stimulation of a cutaneous nerve. Column on the left is at a stimulation rate of 60/min, column on the right is at a stimulation rate of 6/min. Note the appearance of later activity at the slower rate. All traces 5 ms/div. Relative intensities of stimulation from the top down are: 1, 1.1, 1.2, 1.3, and 1.4 on the left, and 1.2, 1.3, 1.4, and 1.5 on the right.







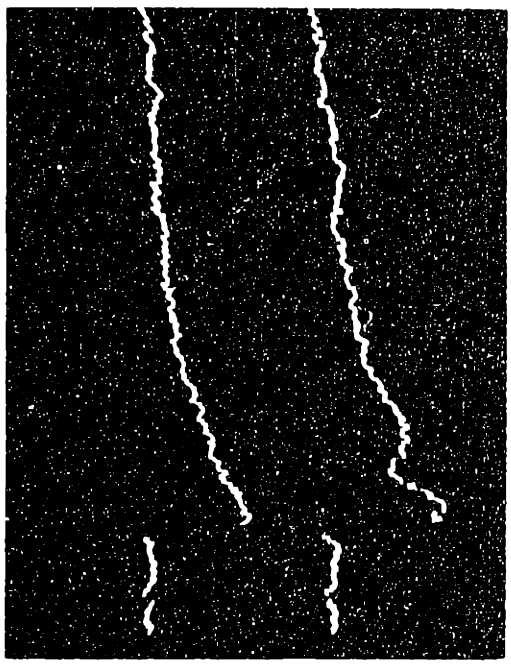
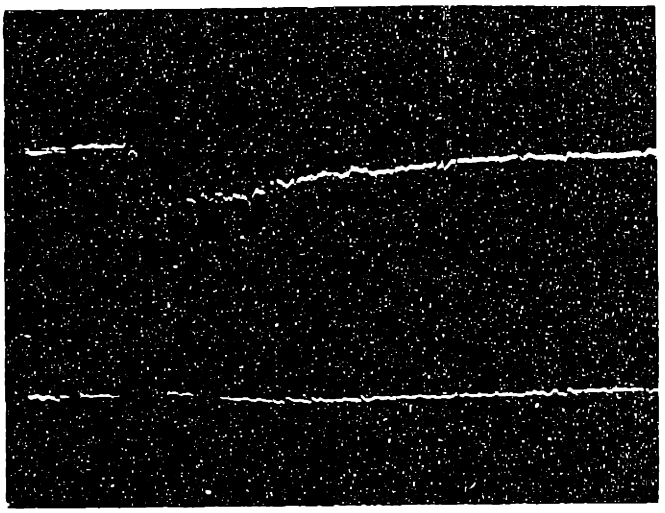
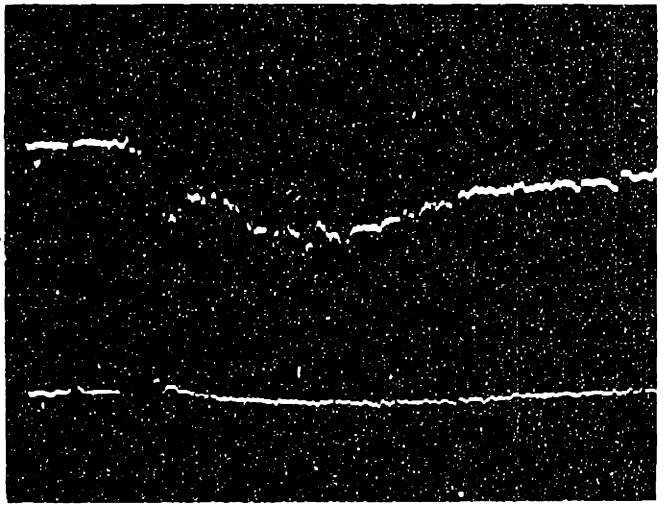


Figure 17 . Another example of the effect of the rate of stimulation of a cutaneous nerve on the dorsal horn focal potential. In all three records the top tract (1 mV/div) is the focal potential, and the bottom trace (500 μV/div) is the record from 9th dorsal root. In the top and middle records the time scale is 5 ms/div. In the bottom record the time scale is 10 ms/div. Stimulation rate 60/min in top record and 6/min in middle record. Bottom record shows superposition of the two responses. Note increase in neuronal discharge at the slower rate of stimulation.





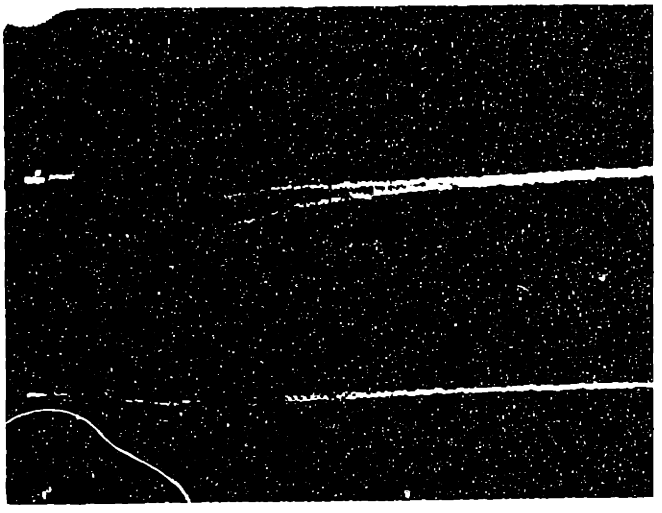
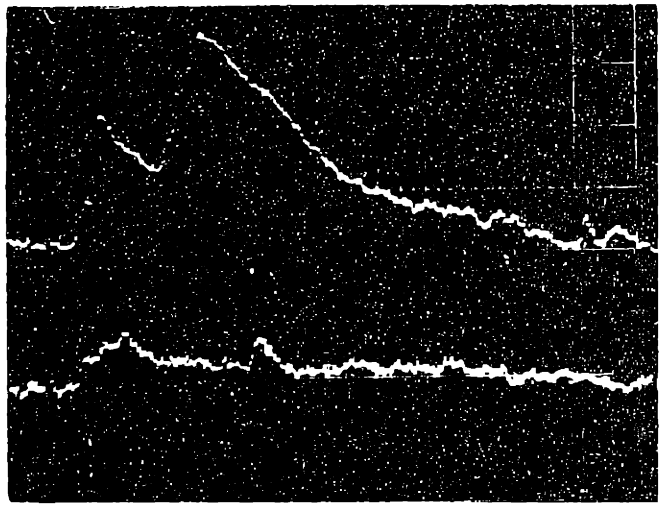


Figure 18a . Intracellular response to maximal stimulation of cutaneous nerve. Top trace, stimulation rate of 6/min; bottom trace, stimulation reat of 60/min. "Flexor" motoneuron with 80 mV resting potential. Both traces at 1 mV/div and 10 ms/div.

Figure 18b . Another example of the effect of stimulation rate on the synaptic activity resulting from cutaneous nerve stimulation. The upper traces are intracellular records. Stimulation rates as described above. The lower traces are recordings from the 9th dorsal root. All traces at 20 ms/div.



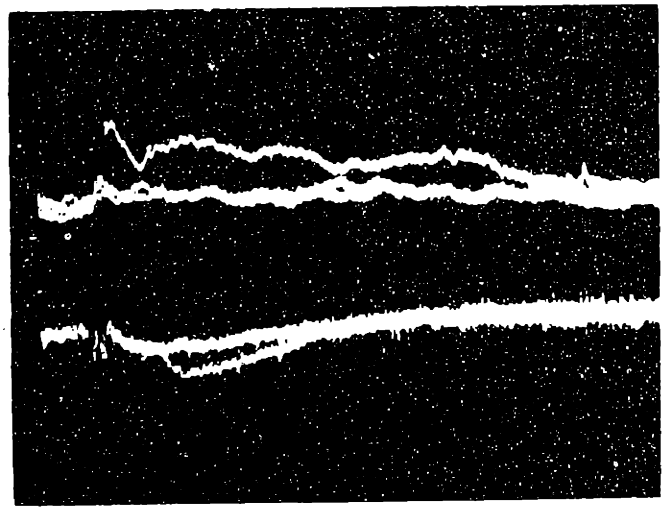
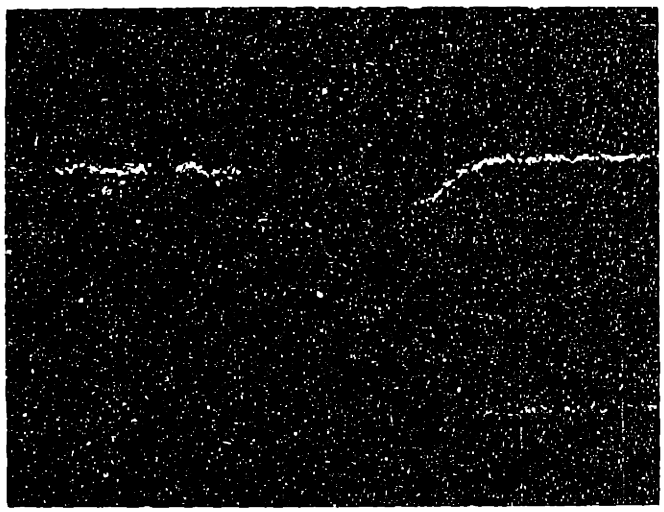
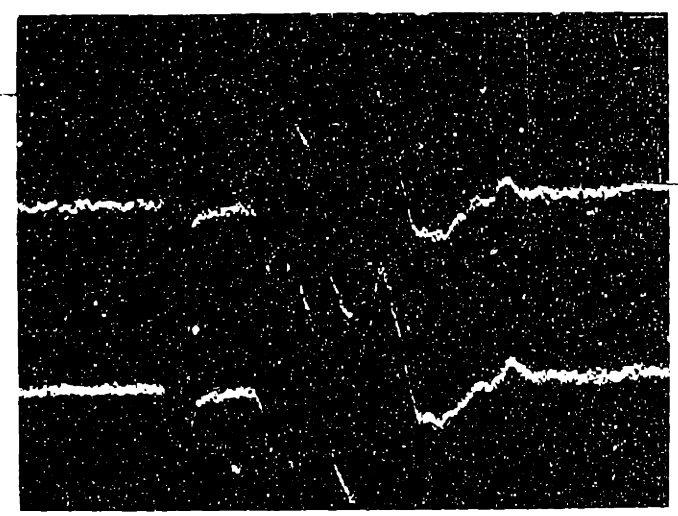


Figure 19a . Monopolar recording on sciatic nerve of the compound action potential resulting from stimulation of cutaneous nerve. Top trace, 5 responses at a stimulation rate of 6/min; bottom trace, 5 responses at a stimulation rate of 60/min. Both traces at 125 μV/div and 1 ms/div.

Figure 19b . Similar to Fig. 19a but with a greater stimulus intensity.

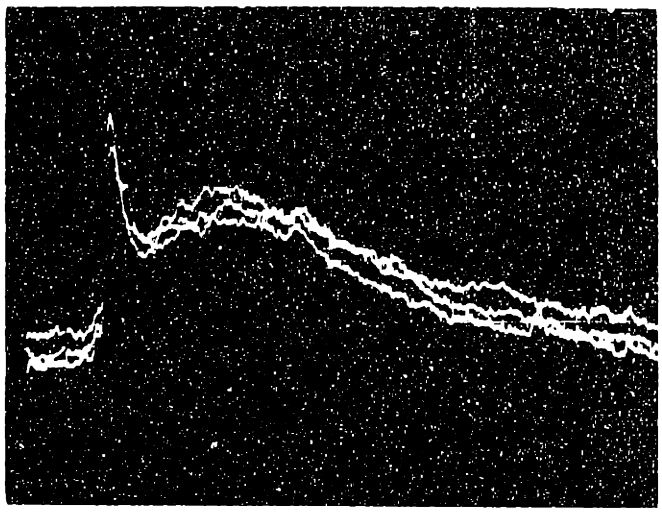


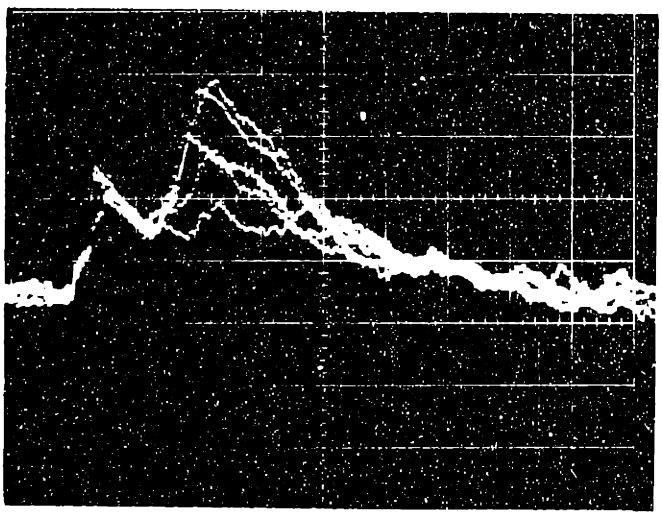


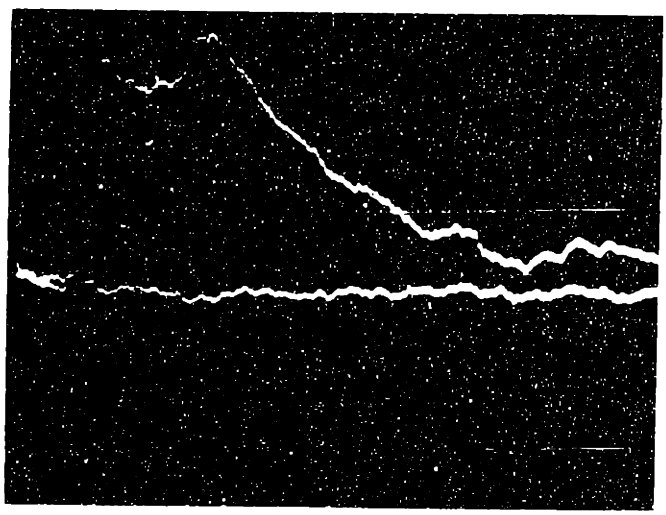
of the earliest PSP's, but they, too, were generally not present at 60/min. The form of the PSP complex response to maximal cutaneous nerve stimulation at 6/min was most frequently a double-peaked depolarization (Fig. 20), but variations were also numerous. The three most common variations, in order of their prevalence, were the absence of a second peak, the replacement of the second depolarization by a hyperpolarization and an amorphous hyperpolarization. Attempts were made to associate the type of response with motoneurons of R. prof. ant. or R. prof. post., but no consistent associations could be made. The only trend that could be detected was that "flexor" motoneurons showed less variation from the double-peaked depolarization than did extensor motoneurons.

The compound action potential recorded from the dorsal root showed at least four conduction velocity groupings for maximal stimulation of the myelinated cutaneous fibers (Maruhashi, Mizuguchi and Tasaki, 1962). The extent to which unmyelinated C fibers were activated was probably small since stimulus intensities above 10X threshold for the largest cutaneous afferents were not used. Attempts to relate portions of the complex PSP to the various conduction velocity groups by varying the simulus intensity indicated that the two fastest groups could account for the general shape of the complex PSP. By further varying the intensity a significant activation of the fastest group could not be achieved alone, since the threshold difference between the two fastest groups was not sufficiently great. The relatively small size of the frog makes it extremely difficult to consistently achieve success with differential blocking techniques. However, it does appear that the fastest group can account for most of the first peak in the double-peak depolarization response. Although cutaneous nerve stimulation at the slow frequencies produced long-lasting synaptic activity,

Figure 20 . Three sets of records from three motoneurons to illustrate the double-peaked depolarizing response to stimulation of R. cutan. dorsi pedis lat. at 6/min. All traces at 10 ms/div. Middle set of records, from the same cell as in Fig. 18a, illustrates the variation seen in the second peak in 5 consecutive sweeps. Bottom set of records illustrates intracellular and extracellular responses.







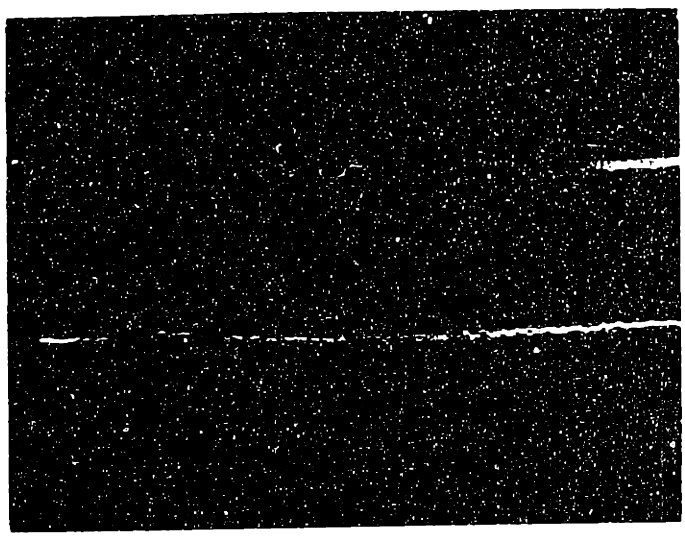
the magnitude of the peak of this activity was seldom greater than 8 mV, and discharge of the motoneuron was an unusual occurrence.

When the rate of stimulation of the muscle nerves was decreased from 60/min (see Results, Part I) to 6/min, polysynaptic activity appeared (Fig 21a,b). Stimulation at 6/min sometimes resulted in an increase of up to 20% in the size of the monosynaptic component of the PSP complex. Of particular interest was the common occurrence of hyperpolarizing responses which had been so conspicuously infrequent at the frequency of 60/min. No detailed investigation was undertaken of the relation between the several types of polysynaptic activity and the peripheral and conduction velocities of the inputs. However, hyperpolarizing responses to "antagonist" muscle nerve stimulation did now occur. Also, stimulation of the contralateral homologus muscle nerve at 6/min resulted in a hyperpolarizing response (Fig. 22) in contrast to stimulation at 60/min which usually resulted in little or no PSP activity.

In summary, even at the slow frequencies optimal for PSP activity, electrical stimulation of peripheral cutaneous or muscle nerve was not, in general, effective in producing sufficient PSP activity to bring the motoneurons to their threshold for discharge. Electrical stimulation of the entire sciatic nerve, or of a dorsal root, does result in motoneuronal discharge (cf. Brookhart, Machne and Fadiga, 1959), but in these cases a far larger number of input fibers are activated than in the situations reported above. Brookhart and Kubota (1963) have reported that electrical stimulation of one relatively select input--the lateral column--resulted in motoneuronal discharge in the excised spinal cord preparation. Selective stimulation of the lateral column is technically difficult in the intact preparations, because size and space restrictions compound the problem of

Figure 2/a . Effect of stimulation rate of muscle nerve R. prof. post., branch descendens communis on the dorsal horn focal potential. Stimulus intensity was 2.1 times the threshold for the earliest response as seen on the 9th dorsal root (lower traces). Upper traces are two superimposed focal potentials. The trace with only the earliest activity corresponds to a stimulation rate of 60/min; the other trace with the additional later activity corresponds to a stimulation rate of 6/min. Top traces at 1 mV/div. Time scale 10 ms/div.

Figure 2/b . Intracellular and extracellular responses to stimulation of R. prof. post., branch descendens communis at different stimulation rates. All traces at ~1 mV/div and 10 ms/div. Cell not identified as a motoneuron by available antidromic stimulation but located in the motoneuron pool. Resting potential 65 mV. From the top down, the responses and stimulation rates are: Intracellular 6/min; intracellular 60/min; extracellular 6/min; extracellular 60/min. This effect of frequency on polysynaptic activity was seen also with R. prof. ant. but not as strongly; the difference may be due to the fact that R. prof. post. has a small cutaneous component.



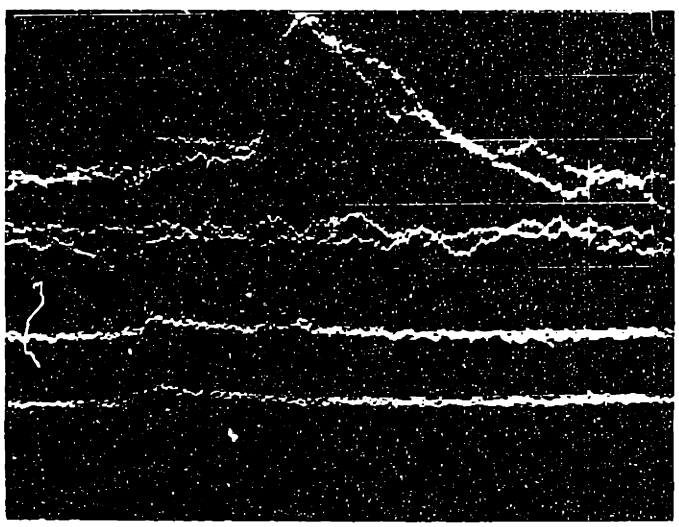
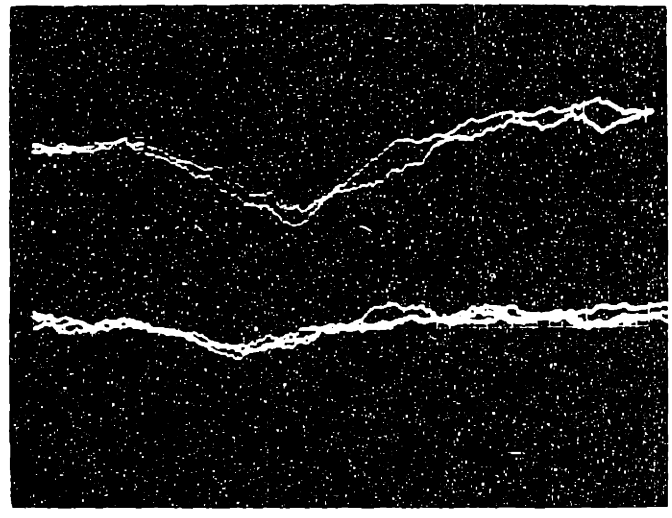


Figure 22 . Effect of the rate of stimulation of the contralateral homologous nerve on the synaptic activity. Intracellular response of a motoneuron of R. prof. post. Top traces are at 6/min; bottom traces are at 60/min. AC recorded at 2 mV/div and 10 ms/div.

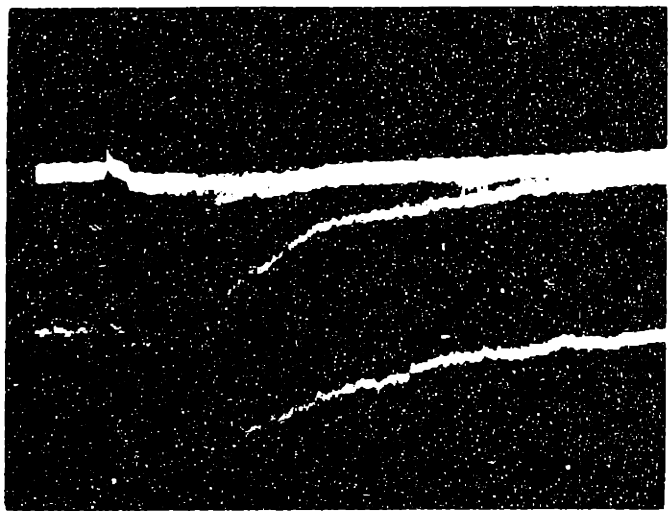


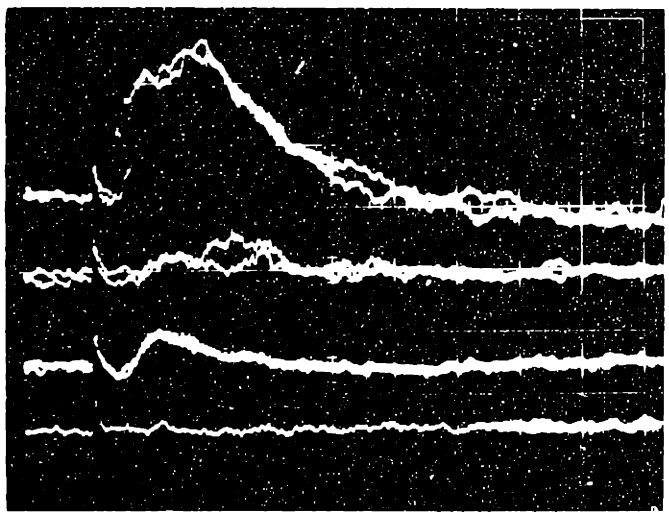
correctly placing the electrodes. Since the lateral column is probably a descending tract from the brain, activation of the several descending tracts at the level of the medulla was done in an attempt to find an input which would cause the motoneurons to discharge. The few experiments that were performed using medullary stimulation were considered only exploratory and are aside from the main concern of Part II, which is an examination of peripheral sensory inputs. Stimulation in the medulla was achieved by using a large (200μ tip) bipolar electrode inserted at the level of the IX and X cranial nerves (glossopharyngeal and vagus). As would be expected from such a gross stimulation in an area as highly differentiated as the medulla, there was a wide variety of synaptic activity produced in the motoneurons. But the result of present interest is that the effect of frequency of stimulation on the production of PSP activity was, in many cases, consistent with that found for peripheral inputs. An example of a case in which abolition of a complex PSP occurred when the frequency was changed from 6/min to 60/min as shown in Fig. 23a,b. In other cases only the later portion of the activity disappeared at 60/min. Also, stimulation in the medulla was more effective than stimulation of the peripheral nerves in producing synaptic activity that caused the motoneuron to discharge. Little can be said as to the correspondence between the effect of medulla and lateral column stimulation. However, the extracellular potential in the ventral horn resulting from medulla stimulation usually had the same polarity and form as the extracellular potential found by Brookhart and Fadiga (1960) for lateral column stimulation. In contrast, cutaneous or muscle nerve stimulation results in an extracellular potential in the ventral horn having a polarity opposite to that seen with lateral column stimulation.

Four chronically spinalized animals were studied to try to determine

Figure 23a. Effect of rate of stimulation of lateral medulla (near entrance of vagus) on the ventral horn focal potential. Traces showing activity correspond to rate of 6/min; traces showing little or no activity correspond to rate of 60/min. 10 ms/div and .5 mV/div.

Figure 23b. Effect of rate of stimulation of lateral medulla on the intracellular and extracellular responses for a motoneuron (resting potential 75 mV). A different preparation than above. Time scale 20 ms/div. From the top down the type of response and the stimulation rate are: Intracellular, 6/min; intracellular 60/min; extracellular 6/min; extracellular 60/min.





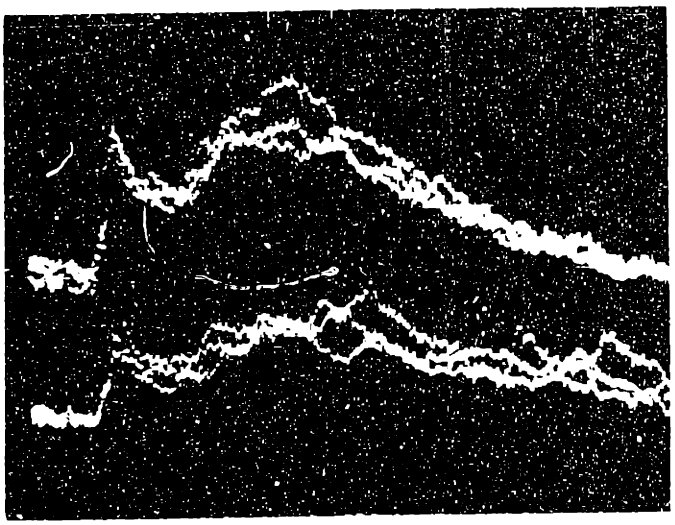
if the processes which silenced the PSP activity at frequencies of stimulation of around 60/min were inherent in the spinal cord. Although the complex PSP response to cutaneous activity was still less at 60/min than at 6/min, it was apparent that the suppression was significantly reduced (Fig. 24).

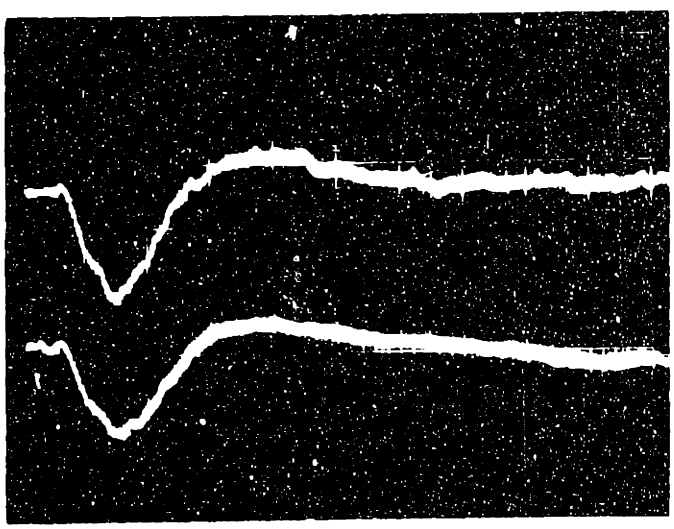
Natural Cutaneous Stimulation

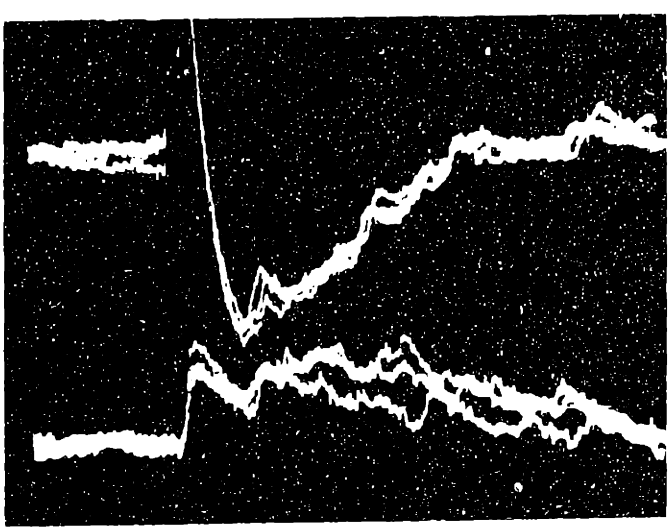
Although electrical stimulation of cutaneous nerves was found to be strikingly incapable of producing motoneuron discharge, such was not the case for natural cutaneous stimulation (cf. Matthews, 1966). The effects in motoneurons of cutaneous stimulation, whether electrical or natural, have always been more difficult to present concisely than have effects of stimulation of the group I muscle afferents. The reasons for this are at least twofold. First, the cutaneous input can be subject to extensive neural transformation in interneuronal paths before it acts upon the motoneuron. The group I muscle input has a more direct representation on the motoneuron via monosynaptic or disynaptic paths. Secondly, or really as a corollary, electrical stimulation of group I muscle afferents can produce input patterns more like the natural input than it can in the case of cutaneous or extero-receptive inputs. Consequently, the results of studies with natural cutaneous stimulation often take the course of examples and impressions; this work is no exception.

The natural stimulation of a brief light punctate touch was found to exert a much more pronounced effect on motoneurons than any form or combination of electrical stimulation of cutaneous nerves. This contrast was most dramatic when first seen in those motoneurons that exhibited a double-peaked PSP complex in response to electrical cutaneous stimulation. When a light touch was applied to the skin served by R. cutan. pedis dorsi lat., these

Figure 24. Three sets of records illustrating the absence of the frequency effect (see text) in chronically spinalized animals. All records are intracellular in three different motoneurons. Top records, response to stimulation of cutaneous nerve at 6/mm (top) and 60/min (bottom). 10 ms/div and ~.6 mV/div. Middle records, response to stimulation of cutaneous nerve at 6/min top and 60/min (bottom). AC recorded at 50 ms/div. Bottom records, responses to stimulation of R. prof. post., branch descendens communis, at 60/min. Top trace, slightly higher stimulus intensity than bottom trace results in antidromic spike. Polysynaptic activity appears in spinalized animal, but in intact animal only monosynaptic component appears at this frequency of stimulation, 10 ms/div and 2 mV/div.





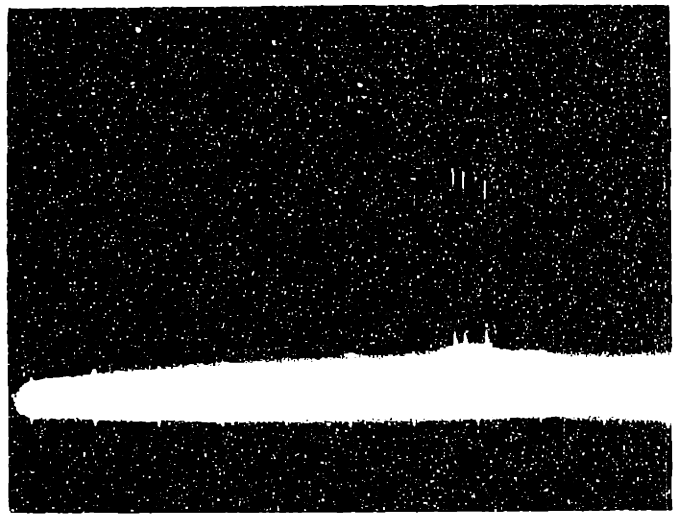


motoneurons often showed much synaptic activity leading to a burst of 3-4 spikes (Fig. 25). Thus, the selective activation of probably only a few cutaneous fibers was far more effective in exciting these motoneurons than was the synchronous activation of a much larger number of cutaneous fibers. While trying to elicit a response in motoneurons by touching different portions of the skin innervated by R. cutan. pedis dorsi lat., it became evident that only certain "spots" were effective. This finding is consistent with the work of Cronly-Dillon (1962) on the types of cutaneous fibers in frogs. The interval required between touches in order to elicit a second synaptic response in the motoneuron was rather variable, but in most cases it was longer than 5 seconds. In general, when the skin was touched with a blunt object ("pressure" stimulation), little or no synaptic activity was produced even with quite large pressures (Fig. 26).

Just as electrical stimulation of the cutaneous nerve resulted in varying combinations of depolarizing and hyperpolarizing responses, so, too, did punctate touch of the skin. In some motoneurons this natural stimulation resulted in a long-lasting inhibition (Fig. 27); in other motoneurons it resulted in a hyperpolarization followed by a depolarization, while in still other motoneurons it produced the opposite sequence. In general, the natural stimulation produced larger and longer-lasting synaptic effects than did electrical stimulation. Also, natural stimulation was signularly effective in producing motoneuronal firing. In most cells no obvious correspondence could be found between the general shape of the PSP complex resulting from electrical stimulation of R. cutan. pedis dorsi lat. with that of the PSP complex resulting from natural stimulation of the skin innervated by that nerve.

In a given motoneuron light touch to different places on the animal

Figure 25 . Examples of motoneuron response to light punctate touch on the dorsum of the foot. Top record at 50 ms/div; first spike rises 60 mV above firing level. Bottom record at 20 ms/div is another response to a second touch.



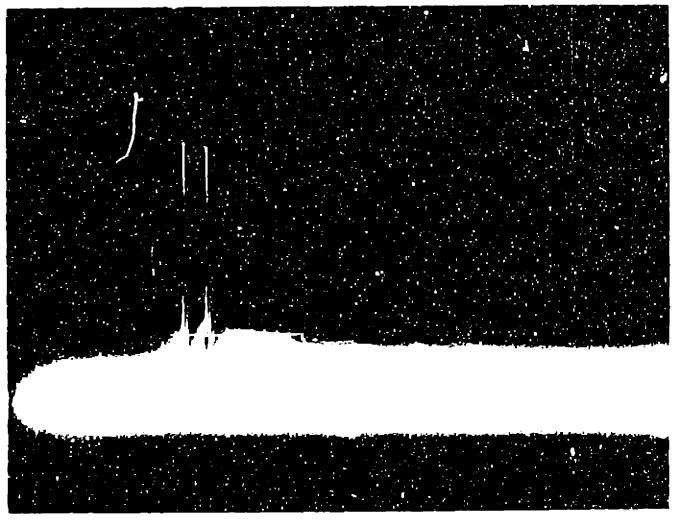
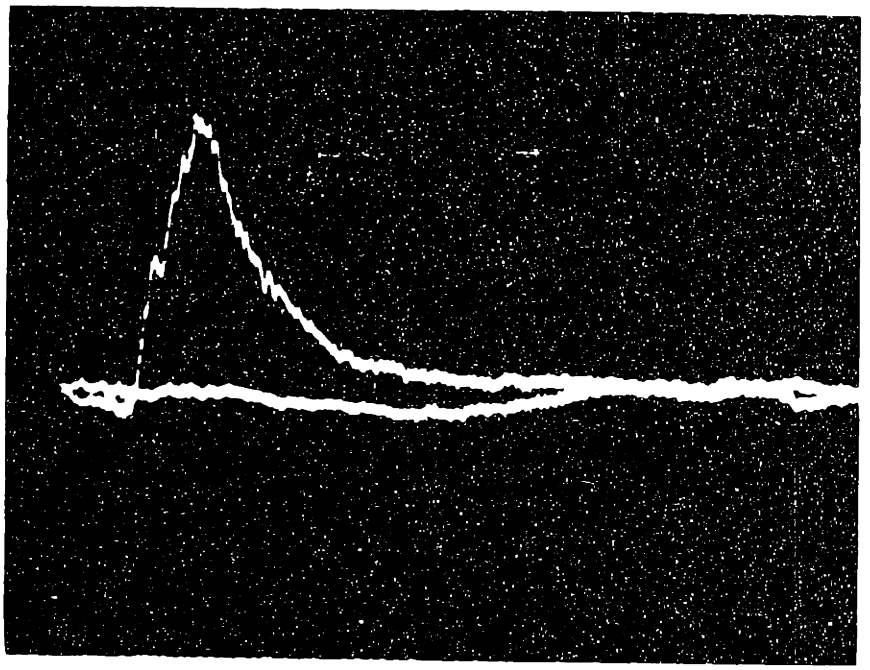
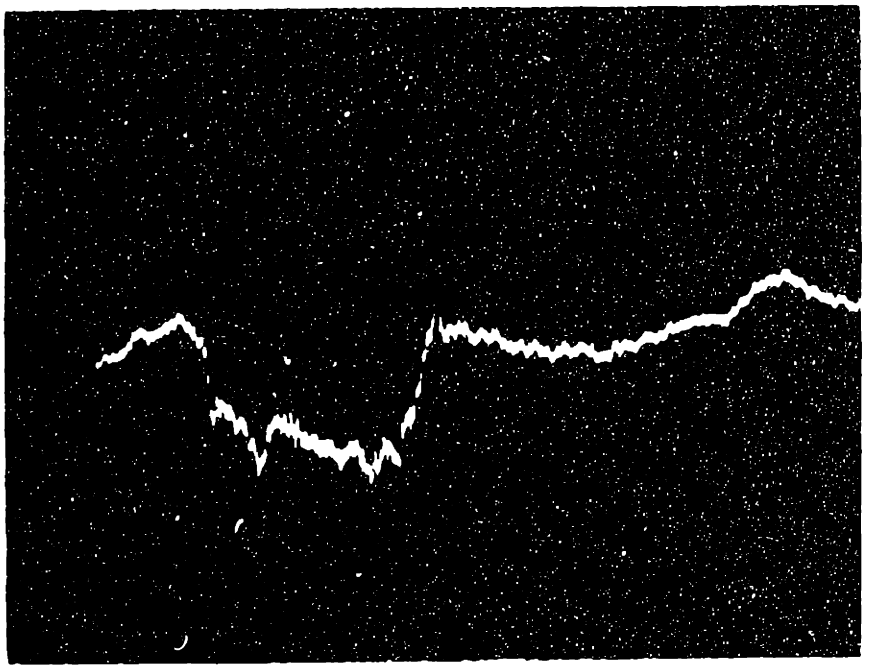


Figure 26 . Difference in effectiveness between light punctate touch and pressure in producing synaptic activity in motoneurons of the twitch motor system. The trace showing synaptic activity is the response to punctate touch on the ipsilateral wrist. The other trace is the response to pressure at the same location. In general, pressure stimuli resulted in little or no synaptic activity in twitch system motoneurons. 200 ms/div; ~.5 mV/div.

Figure 27 . Prolonged hyperpolarization in another motoneuron in response to touch. A single spike occurred at the cessation of the response. 200 ms/div; ~2.5 mV/div.

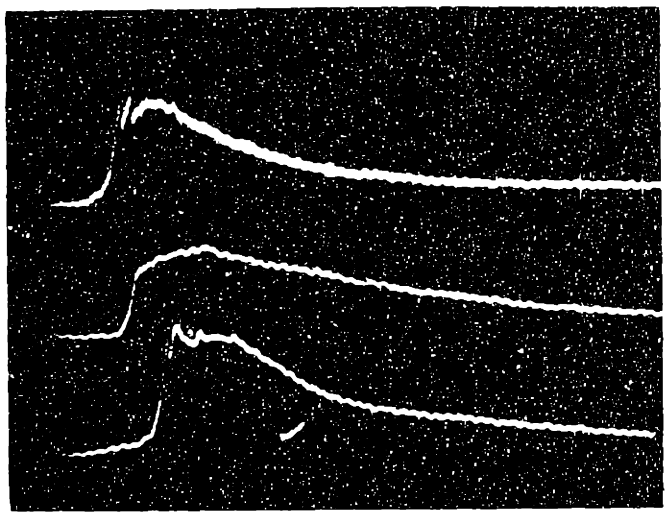


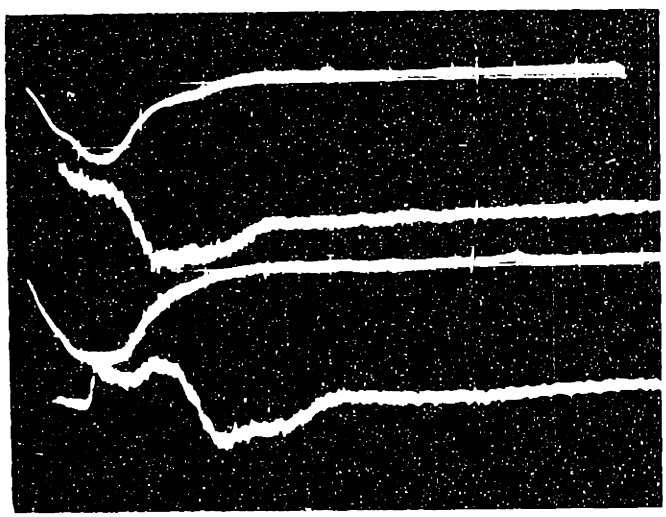


gave very different patterns of PSP activity both with and without firing (Figs. 28a-e). In about one-half of the motoneurons studied the receptive field covered the entire animal, but the pattern of PSP activity changed from place to place. The regions where touch produced motoneuronal firing were most often located near joints or were on the foot. As the location of the "natural" stimulus was moved in small distances (∿ 1 cm) from one presentation to the next, one PSP pattern evolved in a smooth continuous fashion into another (see Fig. 29). One had the impression that one could ascertain a signature of the location of the stimulus by the pattern of the PSP complex. It was as though one was actually seeing "local sign" in the PSP pattern.

This translation of the location of the skin stimulus into a PSP signature could not be obviously extracted in fine detail, but it appeared more grossly in terms of the ordering and duration of the several depolarizing and hyperpolarizing components of the total pattern. It was apparent that tactile stimuli resulted in specific but complex synaptic activity in motoneurons such that, for example, touch on the foot could not be represented simply as excitation of flexors and inhibition of extensors. That is, in the frog the natural cutaneous stimulation of the foot produced more complex PSP patterns than that usually associated with the cutaneous flexor or withdrawal reflex of mammals. As an aside, for the frog, dual extension of the hindlimbs is the response appropriate for comparison to the ipsilateral flexion-contralateral extension response of mammals.

The fact that touch of different skin areas produces, in a given motoneuron, distinct synaptic signatures that strongly effect the neuron's activity suggests that cutaneous sensation has the capability of determining to a significant extent the types of motor behavior seen in amphibians.





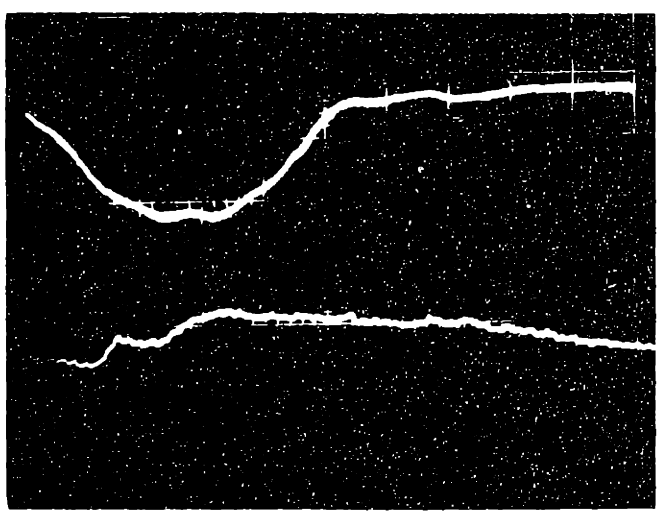
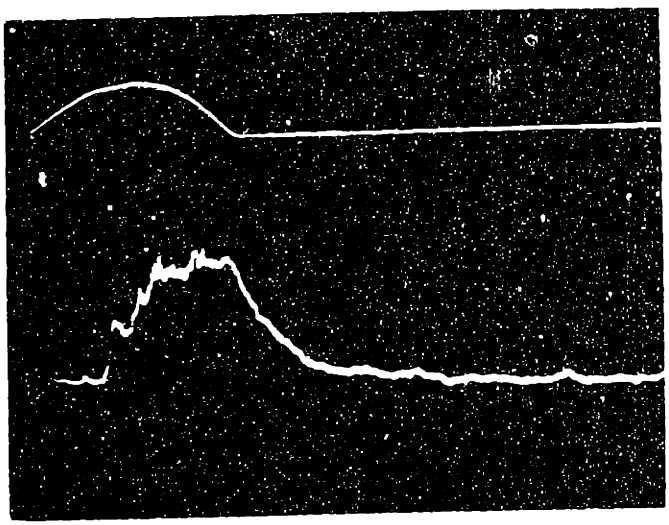
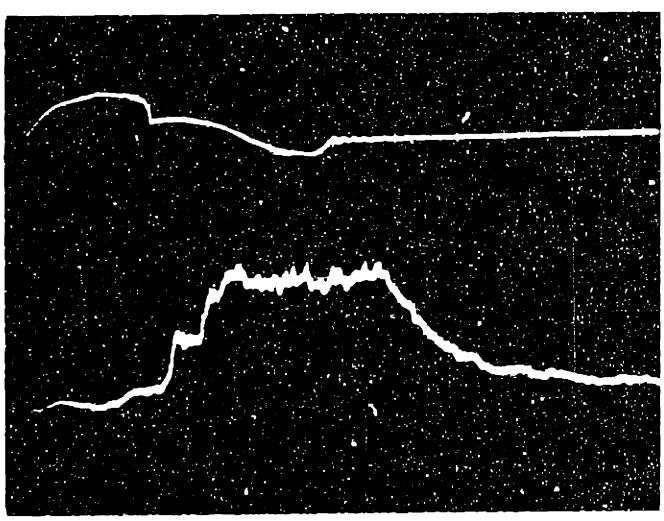


Figure 28c. Examples of the PSP patterns in response to touch at different locations. The responses shown here are from the same motoneuron as in Fig. 29. The site of the touch stimulus was, from the top down: Wrist of ipsilateral forelimb; between elbow and wrist of forelimb; and contralateral ankle. Upper trace in each record is the signal from the strain gage bridge on the touch probe. Lower trace in each record is the intracellular response, ~8 mV/div and 100 ms/div. All responses DC.





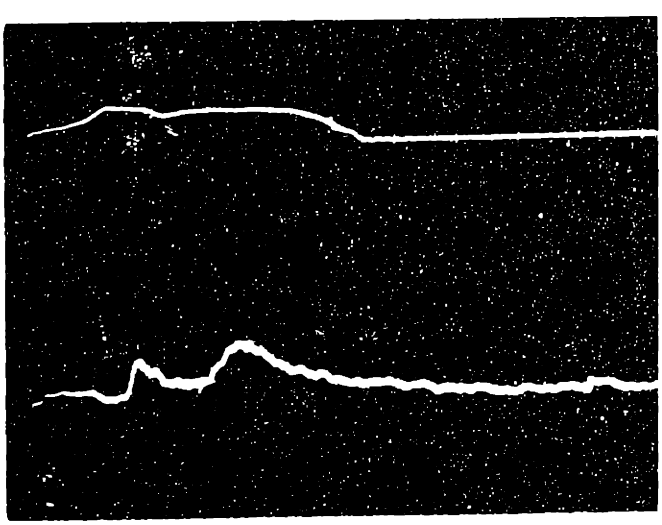
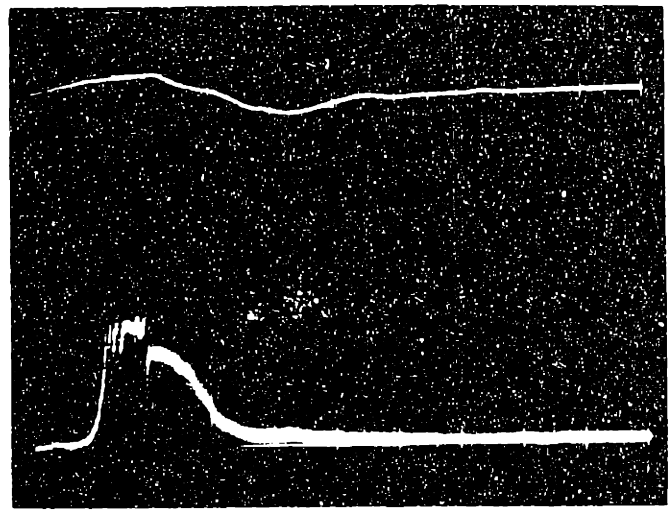
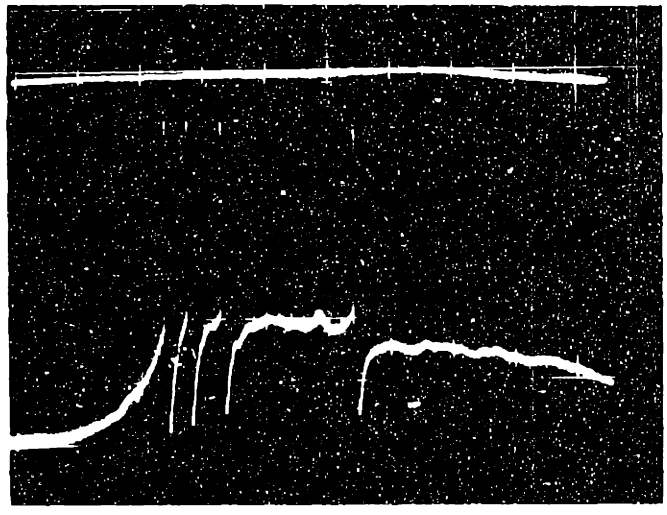


Figure 28d. Examples of PSP patterns in one motoneuron in response to touch at different locations. In each record the upper trace is the signal from the strain gage bridge on the touch probe and the lower trace is the intracellular response. The site of the touch stimulus was, from top down: Ipsilateral ankle; ispilateral ankle; ipsilateral knee. The top and middle records are the same response but at 100 ms/div and 20 ms/div, respectively. The bottom response is at 100 ms/div. All intracellular records DC and at the same amplification.





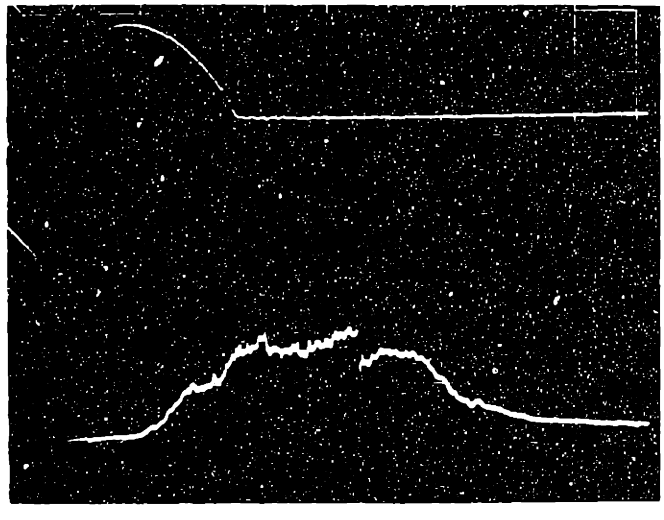
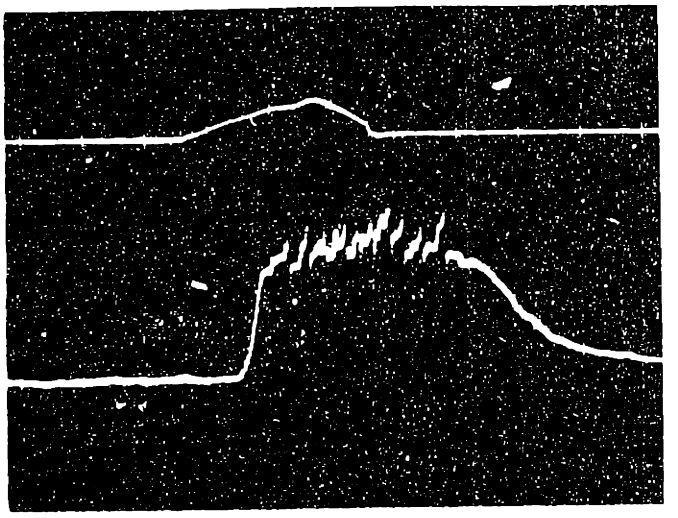
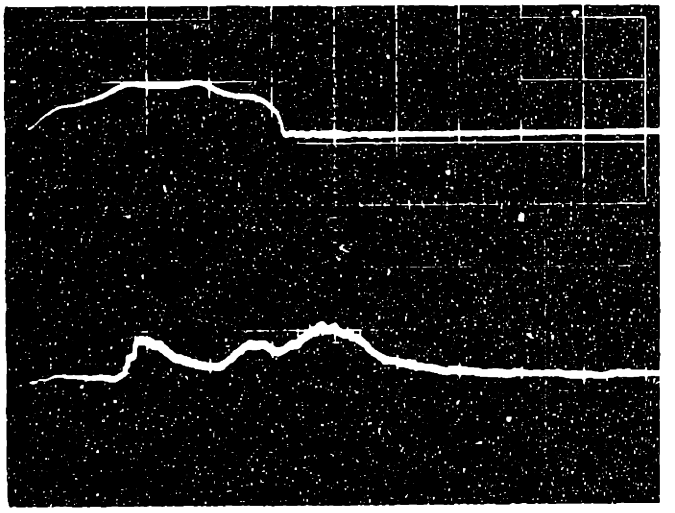


Figure 28e. Examples of PSP patterns in one motoneuron in response to touch at different locations. In each record the upper trace is the signal from the strain gage bridge on the touch probe, and the lower trace is the intracellular response. In the bottom record two responses to the same stimulus are shown, but the resting voltage has dropped from 80 mV (upper traces) to 55 mV (lower traces). Note the effect of reduced membrane voltage on the appearance of the hyperpolarization. The site of tne touch stimulus was, from the top down: Ipsilateral elbow; ipsilateral thumb; ipsilateral knee (two presentations). Time scale is 100 ms/div except for the lower two responses which are at 200 ms/div. All records are DC and at the same amplification.





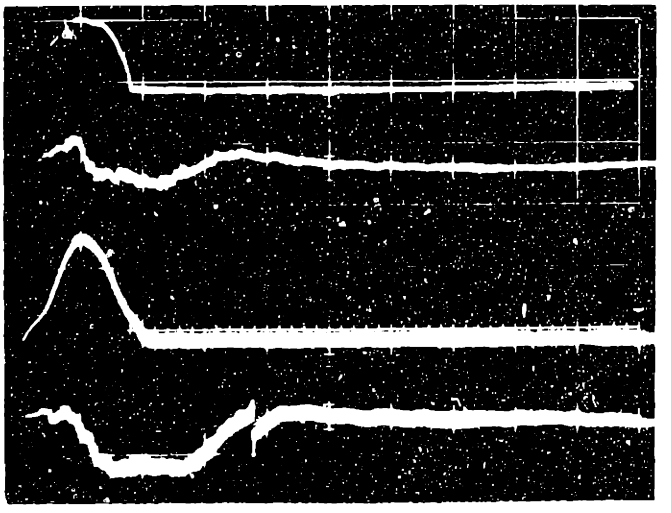
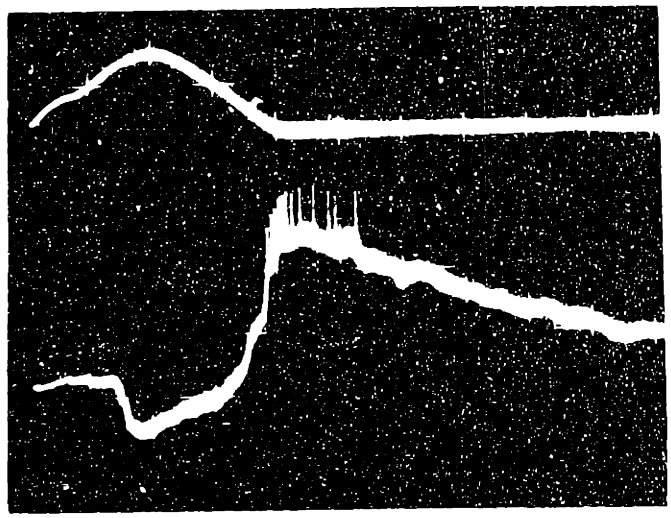
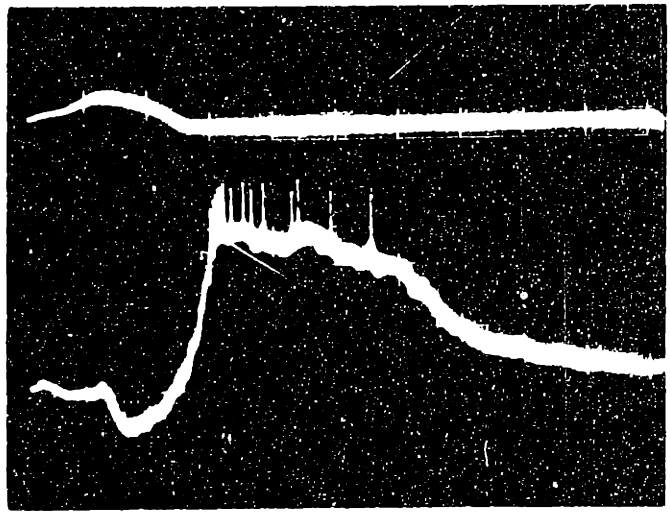
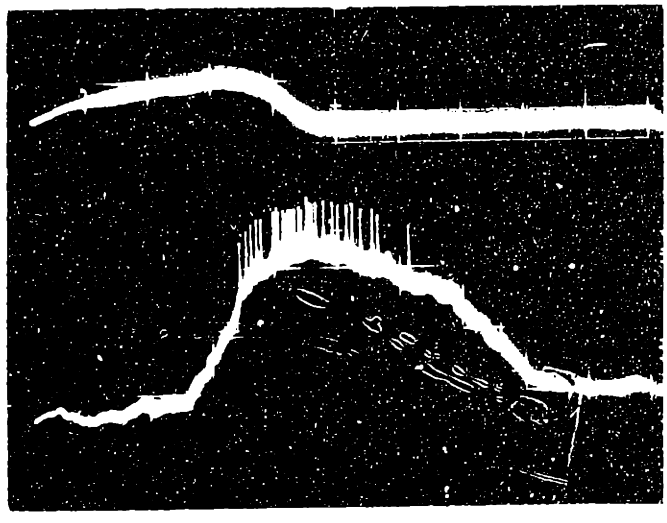


Figure 29. Three records from one motoneuron illustrate the transition of the PSP pattern as the location of the touch was shifted from place to place on the ipsilateral leg. The location of the touch, from top down, was: Ankle; calf; and knee. The upper signal in each record is the output from the strain gage bridge on the touch probe (see Methods, Part II). The lower signal in each record is the intracellular activity in a motoneuron of R. prof. post., ~8 mV/div. Time scale 100 ms/div. Both orthodromic and antidromic spikes did not invade the cell body.







The muscle afferents in frogs have been found (see Part I) to be incapable by themselves of firing the motoneurons. In mammals this is far from the case. But in frogs it appears that cutaneous sense governs the motoneurons far more than muscle sense.

For each motoneuron the differing PSP patterns seemed to reflect at the single cell level the various patterns of motor response which the frog exhibits upon being touched at different places on the skin. In the quiescent frog there are a number of fairly specific non-locomotory motor acts of the hindlimb that can be elicited by light touch to different parts of the skin. These motor responses, which serve to remove the slight irritation by a wiping or flicking or withdrawal, are one-time, explosive-like occurrences, in contrast to the repetitive, more "methodical" scratch response in mammals. Several examples of these phasic motor acts are as follows: A touch on the knee results in a wiping of the knee with the ipsilateral foot; a touch on the lower back results in a wiping of that area with the ipsilateral ankle. Attempts were made to show that in a given motoneuron each PSP pattern, produced by touching different places on the skin, consistently reflected a sequence and intensity of activation which could be associated with some muscle participating in each of the "whole animal" motor responses. Three stimulus locations were used--the ankle, the knee and the lower back--and although each area often produced quite different PSP patterns in any particular motoneuron, there was difficulty in consistently relating the pattern to the supposed role of the "muscle" in each motor act. Actually, for several reasons, the task was too ambitious for the present. First, the two peripheral muscle fibers used to identify the cells as motoneurons each innervate more than one muscle. While R. prof. ant. innervates three muscles that could reasonably be

considered as knee extensor, R. prof. post. innervates muscles that are unlikely to function uniformly. Second, concomitant to the problem of identifying a single muscle type is the problem of not knowing what activity actually occurs in each muscle during the various wiping or withdrawal responses. Because even the simplest responses in the frog occur, not in a plane as for the cat, but in three dimensions, it is more difficult to intuit the manner of participation of each muscle. To determine the role of each muscle in each response would require obtaining electromyograms; this is a separate study in itself. Third, the form of the phasic motor response to tactile stimulation of any given localized skin area (e.g., the dorsal knee surface, the lower back, etc.) depends upon the level of arousal and on the posture. Only when the frog is quiet and in the resting posture can one hope to obtain a consistent stereotyped response to tactile stimuli. Spinalization helps to reduce the variations in response, but even that does not render the response immutable. For example, under resting conditions a light touch in the vicinity of the ankle or dorsal surface of the foot elicits a very brisk extension and return of the lower leg; but if the whole leg is initially swung away from the body, the response will often be simply a withdrawal of the leg to the resting posture. Although one can describe a distinct motor response to the tactile stimulation of a given localized area, it must be remembered that this response is, at best, only the most common of a variety of possible responses peculiar to that specific site of stimulation. For the above reasons, it was not surprising that the PSP patterns in a given motoneuron could not be generally interpreted as reflecting participation consistent with different motor responses to touch. However, it does seem qualitatively evident that cutaneous sensibility plays a far more dominant role than does muscle sensibility in determining motor acts.

DISCUSSION

Part I

The initial aim of these experiments was to see if a measure could be found which would indicate the occurrence of the dendritic resistance transients presumed to be associated with synaptic activity. Although the original goal of the experiments was not in general attained, the negative findings are not without importance for they add further support to the view that any representation of a neuron should provide for the distributed nature of its synaptic input (Rall, Burke, Smith, Nelson and Frank, 1967). The findings also have implications relating to the ways synaptic inputs effect each other when they are combined.

Initially it was hoped that the interaction between the dendritic EPSP and the antidromic M spike, which is the best antidromic approximation to a current source, would result in a decrease in the peak height of the M spike, reflecting the transient decrease in the dendritic membrane resistance. This approach was a new modification of the antidromic spike interaction technique, and was an improvement since it was free from some of the caveats. In general, no decrease in the height of the M spike was found, even with some computer averaging. The findings are similar to the negative findings of Smith et al. (1967) with cat motoneurons in that both results indicate that the peripheral dendritic synapses can produce significant somatic depolarizations at the soma without significantly changing the neuronal impedance as seen from the soma. The present findings are complementary to those of Smith et al.; in the frog the peripheral dendritic origin of the EPSP's studied is known from anatomical (Liu and Chambers, 1957) and physiological (Brookhart and Fadiga, 1960) evidence, while in the cat the EPSP's for which no resistance change was found were

presumed to have peripheral dendritic origins, since their shape indices were consistent with those predicted for remote dendritic EPSP's by Rall's elegant model.

The interpretation of the findings of Smith et al. (1967), published during the course of the present work, supported the conclusions drawn from the present model (see Appendix) that the original aim of the experiment was not likely to be attained. In retrospect the problem was undertaken with a certain naïveté existing as to the relation between the somatic "view" of the effect (depolarization) of dendritic synaptication and the corresponding supposed cause (a membrane resistance transient). That is to say, that initially it was felt that a dendritic EPSP which produced a depolarization of 10-30% of the spike threshold value would be associated with a similarly significant perturbation of the whole neuron impedance as seen from the soma. This line of reasoning indicates retention of the implications of the simple lumped RC neuron model in which the detectability of the resistance change is a direct function of the EPSP amplitude.

After working with the model, it is now clear that the factors of resolution and sensitivity were not sufficiently comprehended at the outset. Although it was apparent that the higher frequencies (1000 Hz) would not be sensitive to remote dendritic resistance changes, the contribution of low frequencies to the sensitivity was overlooked because it was apparent that they would not contribute to the resolution of a brief (1-2 ms) resistance transient. However, the lowest frequencies must "reach out" into the dendrites if the frequencies which represent a compromise between sensitivity and resolution are to be effective. The finding of Kubota and Brookhart (1963) that steady-state hyperpolarization and depolarization did not result in an easily detectable change in height of the dendritic EPSP can be

taken as an indication that the relevant dendritic membrane voltage, which is the driving voltage for the EPSP, was not significantly altered. If a steady somatically-injected current so little effects the dendritic EPSP, then it is reasonable to believe that any test signal coming from the soma, no matter what its resolving ability, will not be affected to an extent above the present experimental threshold of detection. Had this awareness existed at the outset, it certainly would have been very intimidating.

A possible alternative interpretation of the above findings of Kubota and Brookhart draws attention to the practical possibility of discriminating chemical from electrical synapses by detecting resistance transients. The lack of effect of polarization on the dendritic EPSP's could be supposed to indicate an electrical synapse with a relatively high local transmembrane impedance. This type of electrical synapse would act as a local current source, and it would not be effected by the value of the local postsynaptic membrane voltage. By the same token it would not be seen from the postsynaptic side as having any membrane resistance transient. However, at the other end of the spectrum of possible types of electrical synapses are those with a relatively low transynaptic coupling impedance. These types of electrical synapses, if located away from the soma, would be difficult to distinguish from a chemical synapse, since they would both show qualitatively similar responses to electrical attempts to modify their action. In particular, the electrical synapses with a low transynaptic coupling impedance would show an apparent membrane resistance transient, for the impedance changes associated with the presynaptic spike would be "visible" from the postsynaptic cell. Therefore, activity due to chemical synapses, or to some types of electrical synapses, will appear to be associated with a resistance-transient. A discussion of the ways that chemical and electrical

synapses can, theoretically, be distinguished is presented by Rall et al. (1967). They indicate that a principle requirement is electrical closeness between the synaptic site and the measuring site, in other words, a favorable geometry. Returning now to the possible interpretation of Kubota and Brookhart's findings, it seems more reasonable and consistent to consider that the steady polarizing current had no strong effect on the remote dendritic membrane voltage rather than to postulate the existence of a special type of electrical synapse for the dendrites.

The present experiments and the consequent neuron model have more to say about synaptic location effects and the ways in which synaptic activities combine (the integrative properties of the neuron) than they do about distinguishing one type of synaptic mechanism from another. Whatever the synaptic mechanism, a single remote dendritic EPSP appears to the soma essentially as if it were generated by a current source. When the neuron is considered as a geometrical structure rather than as only a simple summing device, there is an increased flexibility of the integrative processes and synaptic location must of necessity be important in determining how synaptic inputs combine. The extent to which two synaptic inputs combine in a non-algebraic manner--the extent to which mutual "shunting" of synaptic activity occurs--depends upon how well each location "sees" the other's resistance and voltage transients. That is, the voltage associated with activity at location X depends upon how activity at location Y modifies the impedance configuration of the neuron as seen from X and, also, upon how the driving voltage at X has been influenced by the activity at Y. Now, in a distributed representation of the neuron a brief resistance transient at X can result in a prolonged voltage transient at location Y from which the whole event looks as though it were due to a current source. Therefore, even

though two synaptic loci are electrically remote in terms of impedance, their combined effects can reflect a significantly greater interaction through the changes in local membrane driving voltage. As the distance between the two synaptic loci is decreased, the impedance interaction can contribute to the non-algebraic summation, but since the resistance transients are shorter lasting than the voltage changes they produce, the non-algebraic effect will still be dominated by the voltage transient. On only the basis of the separation of two synaptic loci no definite statement can be made as to whether their combined effects will be algebraic or not. Even if the two synaptic loci overlap, the combination can be essentially algebraic if the effect of each alone is small. At the level of depolarization associated with the threshold for spike discharge, the effect of somatic synaptic inputs will be reduced while those of the more remote dendrites will be unaltered. Taking the threshold depolarization as about 15 mV, the reduction of a somatic EPSP will be about 20%. Experiments (e.g. of Burke, 1967) in which non-algebraic summation occurred showed reductions of up to 17%, but the usual reduction was only about 4%. In the present experiment interaction of dendritic EPSP's produced by stimulation of different peripheral nerves usually showed algebraic summation (see also Fadiga and Brookhart, 1962). Although insight has been gained as to what factors contribute to the non-algebraic combination of synaptic potentials, an important question remains: What is the significance of the non-algebraic effects? Are they of use in integrative computations, or are they corruptions of these processes that must be circumvented?

In the second part of the Discussion comment will be made on the significance for motor function of the peripheral dendritic location of the monosynaptic input from the muscle sense.

DISCUSSION

Part II

The second part of this intracellular study of frog motoneuron was concerned with the synaptic representation of cutaneous and muscle sense and their respective roles in determining motor function. Previous intracellular investigations of frog motoneurons have largely been done only in the excised spinal cord. Although studies have been addressed to the functionality of peripheral sensation in the frog, they were limited to more gross recording techniques; this report is the first using intracellular methods. Drawing from the numerous intracellular studies of cat motoneurons one can make comparisons between frog and cat that lead to suggestions on how some aspects of motor systems evolved. In discussing the results from single cells in terms of their functional significance, appeal will be made to observations on the "behavior" of animals to indicate a suggested correlation. The degree to which one accepts significance in the parallels to be presented is, of course, determined somewhat by one's own impressions of what different animals are about.

Frequency Effects on Synaptic activity

It has been found that in the intact curarized frog, when the frequency of stimulation of cutaneous or muscle nerve is greater than 20/min, there is little, if any, polysynaptic activity occurring in motoneurons. A similar effect has been found, also, with stimulation at some locations in the medulla. It is believed that this abolition of activity is mainly due to a shutting off of interneurons and that the control responsible for this effect is largely extra-spinal, probably originating in the medulla. The abolishment of the postsynaptic activity is too long lasting (greater than 3 sec) to be ascribed principally to presynaptic inhibition of the type

associated with the dorsal root potential. Also, the earliest components of the dorsal horn focal potential associated with cutaneous inputs were not influenced greatly by alteration of the frequency. But the later component, which was maximal in the intermediate gray was strongly affected and disappeared at 20/min. When a muscle nerve is maximally stimulated at 6/min, the associated dorsal horn focal potential has several components reflecting postsynaptic activity, but when the stimulus is raised to 60/min, it becomes much simplified and appears to be identical with the focal potential found by Fernandez de Molina in cases where Nembutal had been used to suppress interneurons. The monosynaptic component of the postsynaptic activity produced by maximal stimulation of the muscle nerve is somewhat reduced as the frequency is raised to 60/min. It has been shown in Part I that in the intact frog one can use to advantage the affect of frequency of stimulation on interneuron blockage to study only the dendritic monosynaptic EPSP in motoneurons. To achieve this result in isolated spinal cord Fadiga and Brookhart (1960) had to treat the cord with pentobarbital to silence interneuronal activity.

In spinalized frogs the size of the polysysnaptic response undergoes a general diminution as the frequency of stimulations is raised to 60/min, but the dramatic complete disappearance of polysynaptic activity is not seen. This observation argues for a descending affect being responsible for the silencing of interneurons. One's first thought as to the supraspinal region or origin is the medulla and its reticular formation as a source of descending inhibition. In the cat Lundberg (1966) has shown that this area may be so stimulated as to cause extensive reduction in the motoneuronal polysynaptic activity associated with flexor reflex afferents. However, the frequency of stimulation required in the medulla of the cat to obtain partial reduction

of interneuronal activity is more than 100 times the frequency of the frog spinal cord afferent input which generally results in total silencing of interneuronal activity.

A stimulation rate of 60/min is considered to be a slow rate for the cat, but for frog it appears that it is a high rate in that it results in a pronounced shutting down of the spinal cord. One cannot incorporate with very much assurance the affects of the synchronous, unselective input produced by electrical stimulation into a functional picture of the normal frog, but there are some intriguing suggestive correlates with the observed behavior. A frog appears to operate most of the time in a discontinuous manner with motor activity that usually occurs in an explosive or bursting fashion. Of course, the use of the term "continuous" is relative to the scale being employed; the scale being used here is that of time and the unit of time, the second. Using millisecond time, the activity of a cat may appear discontinuous, but in comparison to a frog a cat is "continuous." Although a frog, on land, can walk, it predominantly jumps. In water where the sensory requirements are different from on land, the division between paddling (walking) and jumping locomotion is more even. But whether on land or in water a frog spends most of the time quietly watching; exploration is slight. A frog exhibits no response to a variety of stimuli after the first or second time they are presented if they are not separated by seconds, or tens of seconds. Such adaptation, or habituation, can often be demonstrated in response to touch. It is clear that in response to some natural stimuli the frog does not shut down so rapidly and completely as it does to the electrical stimulation. Perhaps the ignoring of the probably confusing or unspecific artificial input is the most reasonable strategy. The point is that this shutting down affect occurs at an input

interval which is shorter than that to which a normal frog maximally responds. It is suggested that the nervous system of the frog operates on a quite different measure of time than does the cat's. As a consequence, intracellular experiments done with the frog to elucidate function may be difficult to perform because, since the interval between stimuli must be relatively long, the limit of stability of the recording technique is taxed.

A Monosynaptic Reflex?

It has been shown with intracellular recording that the monosynaptic EPSP resulting from muscle afferent stimulation cannot, by itself, bring hindlimb motoneurons of the frog to threshold for firing. This monosynaptic EPSP has size and shape parameters consistent with those monosynaptic EPSP's seen by Fadiga and Brookhart (1960) in response to dorsal root stimulation. In the abducens nucleus of the frog Kornacker (1963) has indicated that small-diameter cutaneous fibers may make monosynaptic connections with motoneuron dendrites. However, since in this study cutaneously produced synaptic activity was in general totally abolished at frequencies where the muscle nerve monosynaptic EPSP was still faithfully following the input, it is concluded here that the cutaneous input to the spinal cord traverses at least one interneuron before it acts upon the motoneuron. It is then reasonable to conclude that the monosynaptic EPSP's seen by Fadiga and Brookhart were produced by muscle afferents. Therefore, as a consequence of the work of Brookhart's group, the monosynaptic EPSP's seen in this study are of peripheral dendritic origin. Other evidence indicating the absence of a monosynaptic reflex in frog and toad has been presented in the Introduction.

The monosynaptic reflex is the neurological correspondence to the observation that stretching of a striated muscle results in the initiation of an opposing contraction. The stretch reflex is handily demonstrated in

the cat; in the toad, using the hindlimb muscles, a stretch reflex is not observed (Mashima, 1955). The results of peripheral studies are consistent with the spinal cord studies; thus there are several types of evidence that the hindlimb motoneurons in frog exhibit no monosynaptic reflex in the classical definition of the phenomenon. However, Holemans, Meij and Meyer (1966) and Meij, Holemans and Meyer (1966) report that a monosynaptic reflex does exist in the South African frog, Xenopus laevis. The results of the present experiments can be used to show that the work of Holemans et al., does not demonstrate a monosynaptic reflex in the usual sense of the term, but, rather, shows the existence of monosynaptic facilitation. They found that only in response to the second of two sciatic volleys did a monosynaptic firing appear on the ventral root. Their plot of the height of this monosynaptic discharge versus the time interval between the paired stimuli was double-peaked with relative maxima at about 6 ms and 25 ms. Now, in the present experiments the most common response to an electrical stimulation of cutaneous nerve was found to be a double-peaked EPSP complex (see Fig. 20). The peaks of this response occur at times very close to the times of the peaks of the plot of Holemans et al. It thus seems clear that their results can best be interpreted as indicating a combining of the monosynaptic effect of the second stimulus with the polysnaptic effect of the first to produce firing of the motoneuron. Thus, the conclusion that the frog and toad do not have an unconditional monosynaptic reflex in their hindlimb motoneuron remains firm. While it is possible that the region of origin of the monosynaptic activity in Xenopus is not as remote as it is in Rana pipiens [see later comparison between frog (Rana) and toad (Bufo)], the present interpretation of the results of Holemans et al. indicates that when the level of synaptic depolarization is near to the firing threshold,

a single synchronized remote dendritic input can result in firing. This observation is not made in any sense as a revelation but simply to indicate that dendritic inputs should not be thought of only as providing a general tonic background depolarization. A dendritic input combined with certain states of synaptic activity can be the immediate synaptic precursor to a neuronal discharge. It is apparent that by comparing, in an otherwise quiescent neuron, the effect of somatic and dendritic synaptic activity, produced by electrical stimulation, one can be misled as to the relative importance of somatic synapses. Since most intracellular investigations are performed, perforce, on animals that are deprived to a large extent of their natural stimulation, it is very easy to get the impression that remote dendritic synapses are relatively ineffective and unimportant. The theoretical work of Rall has done much to caution against this naive view.

Cutaneousness

The intracellular observation that the muscle afferents are quite ineffective by themselves in producing hindlimb motoneuron discharge, coupled with observations that natural cutaneous stimulation produces powerful and location-indicative PSP activity, led to considering the muscle activity of the frog as more determined by senses other than the muscle senses. Previous electrophysiological studies, not on the single cell level, have concluded that the frog and toad are much more reliant upon cutaneous sense than on muscle sense, especially with regard to posture and muscle tone (e.g. Moldaver, 1936; Mashima, 1955; Chambers and Simcock, 1960). These earlier works are confirmed here at the synaptic level. The present results will be used later to suggest how cutaneous and muscle sense are combined to yield a prototype motor system for land use.

Observations and manipulations other than electrophysiological also indicate that the motor function of frog is strongly dependent upon cutaneous input. A general conclusion of naturalists (Cochran, 1961; Noble, 1955) is that the frog exhibits a strong positive thigmotaxis; the frog seeks to have his body surface in maximal contact with objects in the environment, and he takes his orientation to provide extensive tactile contact. In earlier days of physiology, before the wide use of electronic devices and when less sophisticated experiments were done, several observers noted that removal of the skin of one leg of a frog resulted in a marked loss of muscular tone in that leg. Complete flaying of a frog resulted in loss of all tone and rendered the animal motorically inoperative. Comment on the validity of these experiments has already been made in the Introduction.

The study by Mashima on the toad also supported the belief that the movements of frogs and toads are strongly cutaneously determined, since he found that Brondgeest's phenomenon is a tonic skin reflex. This finding is also of some historical interest, since Brondgeest's phenomenon dates from 1860. In essence, Brondgeest's phenomenon is the appearance of a demi-flexion of the hindlimb of a spinalized frog when it is held vertically. Brondgeest showed that this effect was due to a sensory input from the leg and concluded that muscle tone and posture were not of autonomous central origin but were reflexive. This finding was quite significant in 1860 when the spinal cord was often considered to be imbued with much autonomy, even to the point of having a soul (as in the Pflüger-Lotze controversy)! Brondgeest did not specify the origin of the peripheral sensory input required, but after the work of Sherrington on muscle spindles and stretch reflexes in the cat, one would have surmised that Brongeest's phenomenon was due essentially to muscle sensory input. However, Mashima has indicated that such is not the case, but rather that it is basically dependent upon cutaneous sensory input. Actually, this conclusion had been reached much earlier by Cohnstein (1863) on the basis of flaying experiments. However, Mommsen in 1885 found that the muscle input did have a facilitating action for Brondgeest's phenomenon.

It has been the consistent opinion of most investigators who have examined cutaneous and muscle sense in frog and toad, that the cutaneous sense is the far more dominant input for motor functioning. On the other hand, for the cat, which has received much more attention, the opposite case has been put forth. This conclusion may be due in part to the fact that in the cat the muscle sensory inputs have large synaptic effects and, being simpler than cutaneous synaptic effects, are more easily related to observed motor

acts (Eccles, 1953). Also, suppression of skin inputs in the cat by local anaesthetics or peripheral-nerve section does not seriously interfere with much of the cat's motor activity. In response to skin stimulation the cat does have motor behavior beyond the flexor reflex (Hagbarth, 1952; Roberts, 1967), but these have received little attention (Hunt and Perl, 1960) because a simpler input system from the muscle is available for study.

Posture

As indicated in the Introduction, the experiments of Kuffler and Vaughan Williams (1953a, b) have shown that there are two types of motor systems in the frog, the small nerve system and the twitch system. In the cat all muscle fibers exhibit action potentials, while in invertebrates, such as the crayfish, most muscle fibers are of the other type, exhibiting no action potentials. In the frog the muscle fibers that exhibit no action potentials are innervated by the smaller, slower conducting motor axons. The muscle fibers that exhibit action potentials are innervated by the larger, faster-conducting motor axons. Because of the differences in the properties of threshold, tension maintainence and firing duration between the two systems, it has been suggested that the small fiber system may also participate with the twitch system in producing phasic and locomotory activity. In the resting toad only activity in small motor axons is detected (Chambers and Simcock, 1960; Mashima, 1955).

It was pointed out in Results, Part I that the motoneurons which were successfully intracellularly recorded from in this study were of the twitch motor system. Since there are no reports of intracellular recording from motoneurons of the small nerve system, one does not yet know how these two motoneuron populations are similar and different at the synaptic level. If the small nerve system motoneurons have monosynaptic connections with

muscle afferents, then the location of these terminals is probably on the peripheral dendrites, as it is for twitch motoneurons, since the anatomy and the focal potential records show monosynaptic activity only in the dorsal horn. It cannot be said how much more effective the dendritic inputs would be in generating a discharge in these smaller motoneurons (see Kernell, 1966).

With regard to cutaneous inputs, it is suggested that the small nerve system motoneurons receive, through interneurons, an important adequate stimulus from the pressure and tonic touch fibers described for the frog (Cronly-Dillon, 1962). This supposition is made in part out of default, since the twitch motoneurons showed little or no synaptic response to blunt touch or prolonged punctate touch. Small motoneurons must discharge tonically to produce significant tension in their associated muscle fibers, and it is suggested that the tonic activity of cutaneous pressure fibers would be most effective in meeting this requirement. In addition, a frog, as manifesting its thigmotaxis, seeks a posture in which a large portion of its skin is in contact with itself or with environmental objects.

Locomotion

The experiments of Gray and Lissmann on toad have shown that complete deafferentation of one hindlimb hardly interferes with that limb's involvement in locomotion, but does result in noticeable loss in muscle tone during inactivity. More extensive deafferentation results in increasing loss of posture, but the ability to exhibit coordinated quadrupedal locomotion persists, in a distorted fashion, even with only one dorsal root intact. Thus, deprivation of peripheral sensory inputs impedes the phasic motor acts of the frog and toad much less than is the case for mammals. However, even in these amphibians, generation of ambulatory rhythm cannot be generated

by the central nervous system without some peripheral sensory input from at least one dorsal root.

Some of the observations made in this study on the synaptic activity in twitch motoneurons, which serve in phasic and locomotory motor acts, are relevant to these deafferentation studies. It has been shown that in about one-half of these motoneurons tactile stimulation of points widely distributed over the entire body surface produces, through interneuronal paths, synaptic activity that appears to be location-specific or identifying. Gray and Lissmann (1946), working before any of the intracellular work on amphibian motoneurons, concluded that it was the input from the proprioceptors that was required for ambulatory rhythm. However, it has been shown that the proprioceptor inputs by themselves are quite ineffective monosynaptically and have a more limited effect upon interneurons than do cutaneous inputs. Therefore, it seems more likely that the more responsible input is that from the skin which acts through a widely interconnected net of interneurons and motoneurons that can generate from not too specific inputs a basic rhythmic coordination. The recent work of Székely (1968) on amphibian locomotion incorporates his finding of a lack of anatomical grouping of motoneuron cell bodies into several models which show rhythmic, sequential outputs for only quasi-specific inputs.

Muscle Spindles

Kuffler and Vaughan Williams (1953b) have presented analogues between the frog's small nerve motor system and the cat's γ motor system. The extent to which the parallel can be drawn is still not clear, since the properties of the different types of intrafusal muscle fibers in both frog and cat are not consistently agreed upon (Matthews, 1964; Matthews and Westbury, 1965; Smith, 1966). It is agreed, however, that the frog, unlike

the cat, does not have an independent motor system to control the intrafusal fibers. Rather, in the frog the extrafusal and intrafusal muscle fibers are served by branches from one motor axon; this arrangement occurs for both the small nerve and the twitch motor system (Gray, 1957). Although the cat has two anatomically independent motor systems, the γ system for the sensory spindles and the α system for the tension-developing fibers, in many types of motor operations the action of the α and γ systems is found to be co-extensive (Eldred and Hogbarth, 1954). This relation has been called the α-γ link by Granit (1955). An exception to this co-extensiveness appears to be with regard to the monosynaptic reflex where the action of spindle afferents on γ motoneurons is not well understood, but where it is agreed that monosynaptic connections do not exist (Hunt and Paintal, 1958; Eccles, Eccles, Iggo and Lundberg, 1960). It is supposed, therefore, that the branching of a motor axon in the frog represents an elementary form of the α-γ link of the cat. One can only be intrigued by the fact that for the monosynaptic reflex in the cat, which does not exist in frog, the α-γ co-extension also does not exist.

Combinations of Muscle and Cutaneous Synaptic Activity

By considering the synaptic effects of cutaneous and muscle sense inputs on amphibian motor neuron, a picture emerges at the single cell level that is consistant with the observed naturally occurring motor activity. Although in the frog and the toad the hindlimb motoneurons cannot, in general, be fired by the monosynaptic dendritic synapses of muscle afferents acting alone, it is not to be supposed that this input is ineffective for motor functioning. The results of this and other studies have led to the view that the frog's motor functioning is much more determined, or governed by cutaneous sense as compared to the muscle sense. This view,

as presently interpreted, does not imply that monosynaptic muscle inputs are of no importance, but, rather, it means that their participation in controlling the discharge of motoneurons is dependent upon cutaneously derived synaptic activity raising the level of depolarization to near the threshold for discharge. Of course, synaptic activity from other sources, such as the labyrinth, could be effective in providing the necessary additional depolarization, but here we will be concerned specifically with the cutaneous senses. When a variety of synaptic inputs representing different modalities of sensation and having different locations on a neuron combine to produce discharge, no one class of inputs can be said to be responsible; however, the muscle sense inputs, to be effective in firing the motoneuron, are dependent upon the existence of additional inputs.

The dependency of the functionality of muscle monosynaptic activity upon cutaneously generated synaptic activity is taken as a demonstration at the single cell level of thigmotaxis. That is, if the muscle sense is to play a role in determining posture, then the frog must have a reasonable portion of his skin in contact with a surface to provide the necessary cutaneous input. The extent to which cutaneous inputs alone could be translated into posture is not clear, since the synaptic effects of such inputs on a motoneuron of the small fiber motor system is not yet known. The required investigation is hampered by the technical difficulties associated with intracellular recording from small neurons.

In the case of motoneurons of the twitch motor system, the conditions leading to the notion of cutaneous dependency also exist. In fact, the reliance upon monosynaptic muscle inputs may be less in these phasic motoneurons than for posture motoneurons. This supposition as to relative effectiveness for these two types of motoneurons is made on the basis of

several types of indirect evidence. First, it has been shown that the natural input of punctate touch, operating through interneurons, is by itself capable of producing discriminative, long lasting synaptic activity that can fire the twitch motoneurons to presumably give the wiping responses. These wiping responses have a ballistic, preprogrammed appearance when they are freely carried out. Second, the deafferentation experiments of Gray and Lissmann show that sensory deprivation of one leg has a greater effect upon posture than upon locomotion. This differential effect suggests that twitch motoneurons are governed far more through interneurons to which one can more easily assign distributory compensatory features, than by the monosynaptic input. Thirdly, Gray and Lissmann have also shown in a spinalized toad that the monophasic movements, which in sequence comprise the locomotory act, can be elicited in a deafferented limb by tapping the de-efferented limbs. These monophasic responses were those that would be appropriate in an intact animal for simple avoidance of a second additional stimulus. However, Gray and Lissmann have concluded that proprioceptor activity is necessary in order to produce rhythmic ambulation. This conclusion was reached on the basis of the observation that when only one dorsal root was left intact, the corresponding ventral root had also to be intact for rhythmic ambulation. This finding, implying strong segmental organization is rather curious, for it is known (Székely and Czéh, 1967) that any one muscle receives significant innervation via more than one ventral root. Similarly, each motoneuron probably also receives proprioceptor afferents via more than one dorsal root. Therefore, the cutting of the ventral root associated with the one remaining dorsal root would not eliminate proprioceptor inputs in that dorsal root. Gray and Lissmann did not report the effect on the existence of rhythmic ambulation when the ventral root cut was from one or the

other of an adjacent segment. Such types of experiments have to be done before the implication of Gray and Lissmann's work is clear. If, in fact, proprioceptive inputs are required for rhythmic ambulation, their effect is through interneurons and not monosynaptically, for proprioceptor afferents of one root do not distribute throughout the entire spinal cord.

A situation in which the monosynaptic input to twitch motoneurons would facilitate the cutaneous-interneuron pre-programmed activity arises when an external resistance to phasic movement is encountered. In this case, because of the mechanical properties of the spindles and their branched innervation pattern, the afferent input would be greater than normal and would facilitate the motoneuron discharge so as to attempt to overcome the resistance. In other words, the muscle would be presented with a more isometric case than it normally encounters. Mashima has demonstrated such facilitation in comparing isometric with isotonic operation of a muscle. Because of the nature of the innervation of the spindle intrafusal fibers, it is supposed that under light loads or nearly isotonic conditions, the spindle output would be very slight, because only slight differences in length or rate of change of length would then exist between the intrafusal and extrafusal fibers. Compared to the mammalian spindle, little is known about the functional capabilities of the amphibian spindle, and further work is required to elucidate the functional significance of the innervation of extrafusal and intrafusal muscle fibers from the same parent motor axon. Perhaps one approach is to consider the possibility that channeling, or filtering, is occurring at the axonal branch point. (This general idea on information distribution is under current consideration by Dr. J. Lettvin's group.)

As an aside, it is mentioned that anatomically the frog has poorly developed Golgi tendon organs; Huber and DeWitt (1900) stated that the true

tendon organs first appear in the reptiles. In the cat, a function of the tendon organs is to protect a muscle from overload by producing synaptic inhibition of the motoneurons. Although the function of tendon organs in the frog has not been studied, it would be interesting to see if the increased frequency of proprioceptive input associated with an isometric state would, after some time, result in the shutting down of the interneurons through which the required supportive cutaneous inputs act. This shutting down of interneurons has been seen within muscle sense and cutaneous sense, but the possibility of this phenomenon existing across these two types of inputs has not yet been examined.

In symmary, the hindlimb motoneurons of frogs and toads require synaptic activity of cutaneous origin, or of supraspinal origin, in order for the monosynaptic dendritic EPSP to be functionally effective. For the small nerve motor system motoneurons, in their role as determining posture and muscle tone, the tonic cutaneous input is considered to be providing, through interneurons, a depolarization close to or above firing threshold, permitting the dendritic monosynaptic inputs from muscle spindles to be functionally effective. For the twitch motor system motoneurons, the cutaneous input acting through a much interconnected system of interneurons appears to be able to produce a large fraction of the frog's phasic motor repertoire. It is suggested that with regard to twitch motoneurons, the muscle sense inputs enter into motor function more effectively by acting through interneurons rather than monosynaptically. However, as the operating conditions during phasic or locomotory activity become more isometric, the monosynaptic dendritic input can produce significant facilitation.

On Getting Off the Ground

Noting the differences in synaptic location of the monosynaptic muscle afferents, and also the differences in motor ability between frog and cat, I suggest an intriguing evolutionary trend that illustrates one specific functional role of dendritic synapses. In performing the following speculation I am not assigning cause or effect, but only pointing out parallels that seem consistent in several different animals between the synaptic location or organization and the apparent motor abilities.

Dating from the work of Sherrington (1906), the stretch reflex has been considered as a principal determinant of an animal's ability to have a gravity-resistant posture. As this reflex and its afferent source the muscle spindles, were further investigated, predominantly in cats and other mammals, it was found that flexor muscles as well as extensor muscles have a stretch reflex, and the role of the muscle spindle afferents was broadened so that it came to be considered, because of its feedback qualities, as the principal peripheral sense, permitting the performance of complicated motor acts by modulations of them. Other muscle sense organs, such as the Golgi tendon organs, were also found to play a role in motor activity. A picture of posture and locomotion has evolved in which the dominant peripheral sense is the muscle sense; the cutaneous sense is generally assigned a role only in terms of noxious stimuli when it overrides the muscle sense to produce ipsilateral flexion (withdrawal) and contralateral extension. Even though in mammals there are motor responses to non-noxious cutaneous stimuli, hardly anything is known about their central representation on the single motoneuron level (Kolmodin and Skoglund, 1958; Eccles and Lundberg, 1959).

The hindlimbs of the frog (Rana) show no postures or phasic motor acts that could truly be said to represent resistance to gravity; this is reflected

in the absence of a dominant monosynaptic input from the spindles and the consequent absence of a stretch reflex. If a land animal has little resistance to gravity, then almost as a truism, it has an increased area of the body in contact with a surface; if the body surface has tactile sensitivities, then an important source of peripheral information exists. Thus, it seems only reasonable that in the frog a strong cutaneous dependency is found. Even though the efferents from muscle spindles terminate monosynaptically only on the peripheral dendrites of frog motoneurons, they can be functional through combinations of their effects with those of the other motoneuron inputs, both cutaneous and supraspinal,

Now, when an animal has basic posture sets that show an increased ability to operate against gravity, there exists a concomitant reduction in the amount of his body surface in contact with the environment. This development can be imagined to be accompanied by some loss of position-sensitive cutaneous input and to require the existence of a more powerful input from the muscle senses so that the animal is able to function with less dependence upon cutaneous inputs. It might be supposed that although the skin were no longer in contact with the ground, it could have receptors which would provide information about the local surface loadings and deformations associated with different positions of a limb in space. However, it seems unlikely that this information from a surface covering such an inhomogeneous body as an appendage would be sufficiently accurate and unique to provide effective control of the various underlying muscles. The need for more detailed information as to the state of each muscle seems more obvious if finely coordinated motor tasks are to be carried out along with a gravity-resistant posture.

With regard to both the matters of gravity-resistant postures and the

ability to perform complicated, sophisticated motor tasks, the frog (Rana) and the toad (Bufo) show marked differences. The toad, a more terrestrial animal than the frog, is able to support the hind portion of its body well off the ground, and it can walk without dragging any part of its body. The toad often exhibits a stalking behavior in approaching a worm, and in doing so the toad performs discrete, seemingly precise movements of one limb at a time. This food-hunting behavior of the toad appears genteel in contrast to the frog's rather lunging, whole-body movements. There is some neurophysiological evidence suggesting that the monosynaptic input in the toad is less dependent upon the existence of cutaneous inputs for its functionality then is the case for frogs. The intracellular work of Araki et al. (1960) on toad hindlimb motoneurons shows that while the monosynaptic input in toad is similar to that in frog in not being capable of generating a motoneuronal discharge, the monosynaptic EPSP in toad is larger than is the case for frog (cf. Fadiga and Brookhart, 1960). More importantly, in the toad somatically-injected current is effective in changing the size of the monosynaptic EPSP's, whereas in the frog somatically-injected current is ineffective in influencing the size of monosynaptic EPSP's. This fact can be interpreted as indicating that the monosynaptic terminals in the toad are generally closer to the soma than they are in the frog and suggests an anatomical study.

In the cat, which is well able to perform many of its antigravity posture and motor acts without cutaneous inputs, the monosynaptic inputs are distributed widely over the α-motoneuron cell body as well as over the dendrites. Because much of the cat's motor function can be interpreted in terms of the various types of muscle sense monosynaptic and disynaptic inputs onto motoneurons, little attention has been directed on the intracellular level to the more complex effects of cutaneous inputs (Hagbarth, 1952). With frogs

one is forced to consider the combination of the muscle and cutaneous inputs to even begin to relate monosynaptic muscle inputs to motor function.

As one compares animals, it appears that the extent of the locus of the monosynaptic input from muscle spindles shows amovement from the peripheral dendrites toward the cell body which is associated with an ability to exhibit a gravity-resistant posture and to perform more coordinated, continuous motor acts. It is suggested that this increase of distribution from dendrites toward soma is required, perforce, because gravity-resistant postures result in the reduction of reliable cutaneous inputs.

Appendix

A mathematical model of a neuron was constructed to depict the effect of dendritic resistance changes on the somatically-recorded response to a current source applied at the soma (the cell body). Calculations based upon the model give an indication of the feasibility of detecting a dendritic resistance transient through its effect on the magnitude of the M spike.

This model borrows from Rall's early work (1959a,b) on dendritic modeling in which he showed that, with certain restrictions on the dendritic branching geometry, the dendritic tree could be reduced to an electrotonically equivalent cylinder. Rall's early models were based upon solutions to the partial differential equation describing a passive core conductor. The solutions represented separately the cases of somatically-injected current producing polarizations in synaptically quiet cells and the cases of local membrane resistance transients producing PSP's. The present model permits consideration of the interaction between injected current and membrane resistance transients, a situation not represented in Rall's earlier work (1960, 1962). The representation of the interaction is achieved essentially by using a set of boundary conditions different from those used by Rall and by removal of the space dimension, leaving only the time dimension. One consequence of the removal of the space dimension is that the model represents only the response at the cell body. This is not a limitation since an intracellular microelectrode is presumed to be located in the cell body. A second consequence of the removal of the space dimension is that the model permits a current source to act only from the soma. This representation is consistent with the experimental situation since the M-spike current was introduced into the cell via the axon, and in motoneurons the axon joins the cell at,or very near, the soma (Sala y Pons, 1892).

The dendrites are considered to be reducible to an electrotonically-equivalent cylinder of semi-infinite length. That is, they are represented as a type of passive coaxial transmission line. The approximation introduced by using a semi-infinite length rather than a finite length is probably not too serious for the case of frog motoneurons since they have far ranging dendrites (up to 1 mm in length). Also, where comparisons could be made between the present model and Rall's finite model, the discrepancies were only 3-4% and of little consequence for the degree of accuracy required from this model.

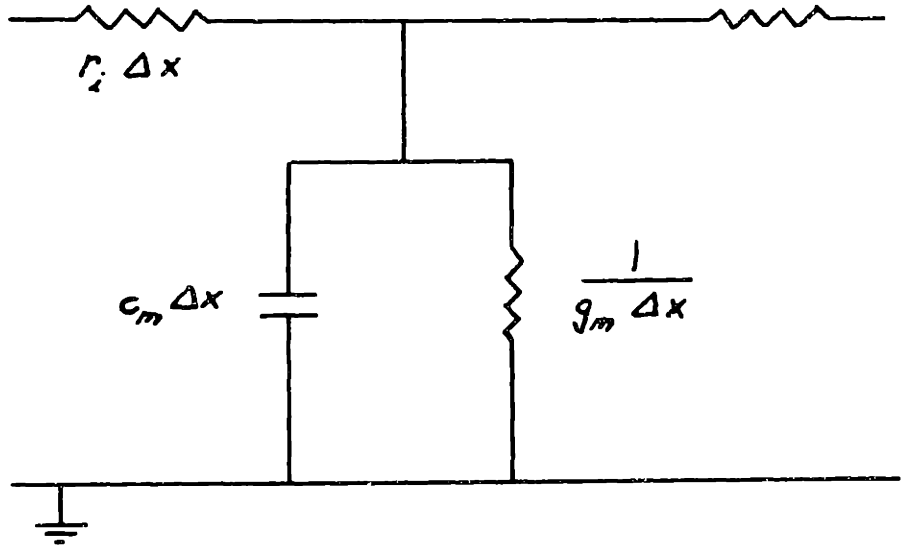
By representing the dendrites as effectively semi-infinite and by restricting dendritic membrane resistance changes to be uniform over the entire dendritic surface, the distributed system of dendrites can be represented by a lumped impedance--namely, the characteristic input impedance Z_c of a semi-infinite transmission line. The properties of the specific coaxial line used are indicated in the equivalent circuit for a segment of the equivalent cylinder (Fig.30). It is assumed that the extracellular path has effectively zero resistivity; the justification, and limitation, of this assumption has been treated by Rall (1959b). The characteristic impedance Z_c for such a coaxial line is

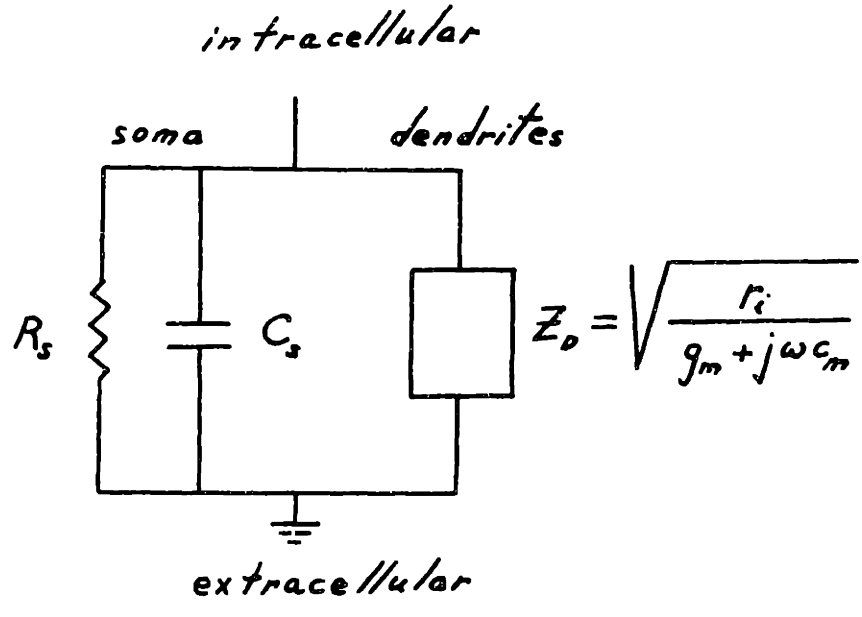
$$Z_c = \sqrt{\frac{r_i}{g_m + j\omega c_m}}$$

where r_i is the core resistance per unit length (Ω/cm), g_m is the resting conductance of the dendritic membrane per unit length (mhos/cm), and c_m is the dendritic membrane capacity per unit length (μF/cm). The soma is represented in the usual fashion as a resistance and capacitance in parallel. The model, thus, consists of two compartments, one with lumped soma impedance Z_s and the other with a lumped representation of the dendrites.

Figure 30. Circuit for a segment of the coaxial transmission line used in representing the resting dendrites.

Figure 31. Circuit representation of the soma-dendrite neuron model.


$r_i \Delta x$
$c_m \Delta x$
$\frac{1}{g_m \Delta x}$


intracellular
soma
dendrites
R_s
C_s
$Z_0 = \sqrt{\frac{r_i}{g_m + j\omega c_m}}$
extracellular

The circuit is pictured in Fig.31. In this form the model is more akin to Rall's later (1964) representation of the neuron--the quasi-distributed compartmental model--than it is to his earlier, more analytical representations. Although this model incorporates a number of assumptions and approximations, it is suitable for indicating the order of magnitude of the shunting effect.

The impedance Z_R representing the cell in the absence of synaptic activity is found from

$$\frac{1}{Z_R} = \frac{1}{Z_S} + \frac{1}{Z_0} = \frac{1}{R_S} + j\omega C_S + \sqrt{\frac{j\omega c_m + g_m}{r_i}} \qquad (1)$$

where R_s is the soma resistance (Ω) and C_s is the soma capacitance (μF). Now, the synaptic activity is presumed to involve a decrease in the local membrane resistance. In this model the resistance transient will be approximated by a step change occurring in either the soma of dendrites or both. The more general cell impedance (Z) includes two parameters Γ and α to provide for membrane resistance changes representing synaptic activity in soma and dendrites respectively. Then

$$Z = \frac{R_s}{\Gamma + j\omega C_s R_s + R_s\sqrt{\frac{j\omega c_m + g_m}{r_i}}} \qquad (2)$$

where Γ reflects the intensity of somatic synaptic activity and α reflects the intensity of dendritic synaptic activity. Both Γ and α are equal to 1, for no synaptic activity and are increasingly greater than 1 for increasingly more intense synaptic activity.

Another parameter ρ, of use when considering dendritic versus somatic

contributions, is the ratio of the real part of the resting somatic impedance to the real part of the resting dendritic impedance for $\omega = 0$.

$$\rho = \left(\frac{R_s}{R_d}\right)_{\substack{\omega=0 \\ \Gamma=\alpha=1}} = R_s\sqrt{\frac{g_m}{r_i}}$$

The limiting value $\rho = 0$ represents a soma without dendrites, while the limiting value $\rho = \infty$ represents a negligible soma. Thus, the larger ρ is, the greater the possible contribution or dominance by the dendrites.

Introducing ρ and τ into (2) results in an expression for Z that more clearly reflects the relative contribution of the soma and dendritic compartments

$$Z = \frac{R_s}{(\Gamma + j\omega\tau) + \rho\sqrt{\alpha + j\omega\tau}} \qquad (3)$$

What is of interest is how strongly changes in Γ and α influence the shape of the voltage transient that results from a current source applied to Z. It was assumed that the current source associated with the rising phase of the M spike could be satisfactorily approximated by a current step since the higher frequencies of the step, being mainly capacitative current, are relatively ineffective in indicating a change in peripheral dendritic resistance. The manner in which frequency enters into a trade-off between sensitivity and resolution with regard to detecting transient resistance changes is considered in the Discussion, Part I.

The voltage response to a current step applied to Z can be found using Laplace transforms. In fact, since V(0) is zero, the voltage response is the inverse transform of the product of the transform of a step function

times the impedance written in transform notation. Thus,

$$V\left(\frac{t}{\tau}\right) = \mathcal{L}^{-1} \frac{\tau I_0 R_s}{s\left(\Gamma + s\tau + \rho\sqrt{s\tau+\alpha}\right)} \tag{4}$$

By letting $w = s\tau+\alpha$ and then factoring, (4) can be put in a form that appears in standard tables of transforms.

$$V\left(\frac{t}{\tau}\right) = \mathcal{L}^{-1} \frac{\tau I_0 R_s}{(w-\alpha)\left[w + \rho\sqrt{w} + (\Gamma-\alpha)\right]} \tag{5}$$

and by factoring

$$V\left(\frac{t}{\tau}\right) = \mathcal{L}^{-1} \frac{\tau I_0 R_s}{\lambda(w-\alpha)} \left\{ \frac{1}{\sqrt{w} + \left(\frac{\rho-\lambda}{2}\right)} - \frac{1}{\sqrt{w} + \left(\frac{\rho+\lambda}{2}\right)} \right\} \tag{6}$$

where $\lambda = +\sqrt{\rho^2-4(\Gamma-\alpha)}$ and is real for the cases considered here.

Now, by taking the inverse transform of the two terms of (6), the expression for the voltage transient is obtained.

(See page 149 for this expression) (7)

This rather unwieldy expression can be simplified to represent some special cases. An important one is that corresponding to no synaptic activity, $\Gamma = \alpha = 1$.

$$V\left(\frac{t}{\tau}\right) = \frac{I_0 R_s}{\rho^2-1} \left\{ \rho \operatorname{erf}\sqrt{\frac{t}{\tau}} - 1 + e^{(\rho^2-1)\frac{t}{\tau}} \operatorname{erfc}\rho\sqrt{\frac{t}{\tau}} \right\} \tag{8}$$

Eq. 7

$$V(0,\tfrac{t}{\tau}) = \frac{I_0 R_s}{\lambda}\left\{\frac{1}{(\frac{\rho-\lambda}{2})^2-\alpha}\left[\left(\frac{\rho-\lambda}{2}\right)-\sqrt{\alpha}\,erf\sqrt{\frac{\alpha t}{\tau}}-\left(\frac{\rho-\lambda}{2}\right)e^{\left[(\frac{\rho-\lambda}{2})^2-\alpha\right]\frac{t}{\tau}}erfc\left(\frac{\rho-\lambda}{2}\right)\sqrt{\frac{t}{\tau}}\right]\right.$$

$$\left.-\frac{1}{(\frac{\rho+\lambda}{2})^2-\alpha}\left[\left(\frac{\rho+\lambda}{2}\right)-\sqrt{\alpha}\,erf\sqrt{\frac{\alpha t}{\tau}}-\left(\frac{\rho+\lambda}{2}\right)e^{\left[(\frac{\rho+\lambda}{2})^2-\alpha\right]\frac{t}{\tau}}erfc\left(\frac{\rho+\lambda}{2}\right)\sqrt{\frac{t}{\tau}}\right]\right\}$$

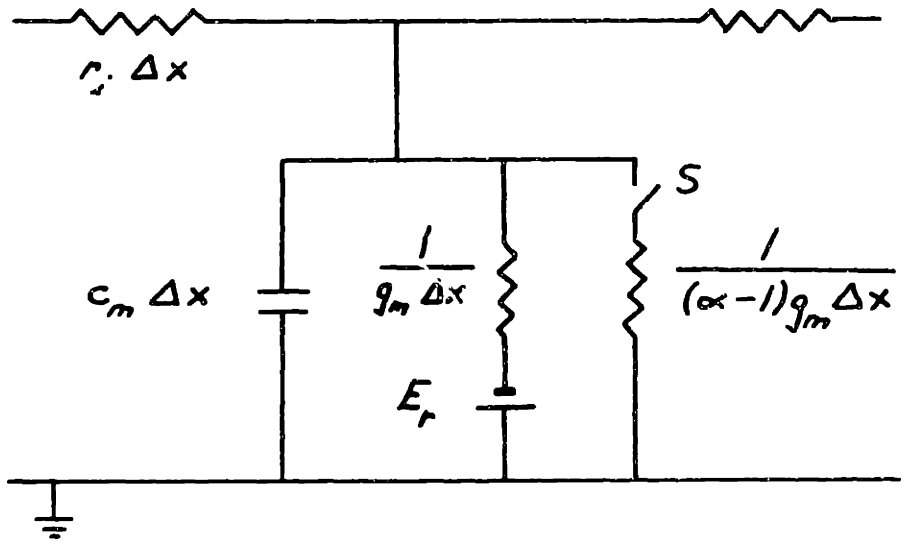
This expression represents the response of the resting cell to a current step I_o, and this is the reference voltage transient. Comparison of Eq. (7) with Eq. (8) indicates how strongly various membrane resistance changes mofidy the shape and size of the voltage response. Equation (8) was also obtained by Rall but via the route of the partial differential equation obtained by considering the unit equivalent circuit of Fig.30 as a differential element. In the steady state, $t/\tau = \infty$, Eq. (8) reduces to

$$V(\infty) = \frac{I_o R_s}{\rho + 1} \tag{9}$$

In order to determine the value of α and/or Γ that would correspond to the experimental situation, the model was expanded to include the production of a membrane depolarization from a step change in α and Γ . The depolarization onset was considered as an approximation to the rising phase of the actual EPSP. By selecting the α and/or Γ that resulted in an approximate matching of the two EPSP's, a realistic, representative value for α and/or Γ was determined for use in Eq. (7). To accommodate the effect of membrane resistance change on membrane voltage the circuit of Fig.30 was transformed to the usual representation of postsynaptic nerve membrane by the inclusion of batteries (Fig.32). The reversal voltage for the EPSP was taken as 0 mV, and since inhibitory PSP's were not of prime concern, no representation for their production was made. E_r is the resting membrane potential. The partial differential equation governing the voltage depolarization transient produced by the closure of switch S has been developed by Rall (1962).

$$\frac{\partial^2 V}{\partial z^2} - \frac{\partial V}{\partial T} - \alpha V = -\alpha V^* \tag{10}$$

Figure 32. Circuit for a segment of the dendrites with provision for EPSP production. To accomodate the effect of membrance resistance change on membrane voltage the circuit of Fig. 30 was modified to the usual representation of postsynaptic nerve membrane. See Fig. 1


$r_i \Delta x$
S
$c_m \Delta x$
$\frac{1}{g_m \Delta x}$
$\frac{1}{(\alpha-1) g_m \Delta x}$
E_r

V is the membrane depolarization measured from the resting membrane voltage; $z = \frac{x}{\lambda}$ where x is axial distance along the cylinder; $\lambda = \sqrt{\frac{1}{g_m r_i}}$ is the steady-state electrotonic length constant; $T = t/\tau$; $\alpha = \frac{g_m + g_\epsilon}{g_m}$; and $V^* = \frac{-g_\epsilon E_r}{\alpha g_m} = \frac{-(\alpha - 1)E_r}{\alpha}$, the steady-state value of V for an <u>isolated</u> uniform patch of membrane.

Equation (10) applies to the case where the dendritic tree is considered as reducible to an electrotonically equivalent cylinder as presented above. Equation (10) has been solved by Rall for several sets of boundary conditions but not for those conditions describing the model neuron configuration presented here. The boundary condition at z = 0 is the expression of continuity at the soma-dendritic junction and through it, provision is made for synaptic resistance changes in the soma. Thus, at z = 0

$$\frac{\partial V}{\partial z} = \frac{1}{\rho}\left(\frac{\partial V}{\partial T} + \Gamma V - \Gamma V_s^*\right) \tag{11}$$

where $$\Gamma = \frac{G_{SR} + G_{S\epsilon}}{G_{SR}}$$

and $$V_s^* = \frac{-(\Gamma - 1)}{\Gamma} E_r$$

Boundary condition (11) does not provide for injected current at the soma. The second boundary condition is that V remains bounded as z approaches infinity. The initial condition is V(z,0) = 0. The solution of Eq. (10)

is achieved using the methods of Laplace transforms applied to partial differential equations. Taking the transform of Eq. (10) and using the initial conditions, results in an ordinary differential equation

$$\frac{d^2Y}{dz^2} - \alpha Y - sY = -\frac{\alpha V^*}{s} \tag{12}$$

where $$Y = \mathcal{L}[V(z,T)]$$

A complete solution of Eq. (12) is

$$Y = A(s)\sqrt{\alpha+s}\, e^{\sqrt{\alpha+s}\, z} - B(s)\sqrt{\alpha+s}\, e^{\sqrt{\alpha+s}\, z} + \frac{\alpha V^*}{s(s+\alpha)} \tag{13}$$

The boundary condition at $z = \infty$ requires that $A(s) = 0$. By use of the transformed boundary condition at $z = 0$, $B(s)$ is determined

$$B(s) = \frac{\dfrac{-\alpha V^*}{s(s+\alpha)} + \dfrac{\Gamma V_s^*}{s(s+\Gamma)}}{\dfrac{s+\Gamma+\rho\sqrt{s+\alpha}}{(s+\Gamma)}} \tag{14}$$

Since we are concerned primarily with the response at the soma, the taking of the inverse transform can be made simpler by first setting $z = 0$. Then

$$V(0, \tfrac{t}{\tau}) = \mathcal{L}^{-1}\left\{B(s) + \frac{\alpha V^*}{s(s+\alpha)}\right\} \tag{15a}$$

and, after combining with (14)

$$V(0,\tfrac{t}{\tau}) = \mathcal{L}^{-1}\left\{\frac{\alpha V^*}{s(s+\alpha)}\left[\frac{\rho\sqrt{s+\alpha}}{s+\Gamma+\rho\sqrt{s+\alpha}}\right] + \frac{\Gamma V_s^*}{s\left(s+\Gamma+\rho\sqrt{s+\alpha}\right)}\right\} \qquad (15b)$$

After taking the inverse transform, the expression for the depolarization transient at the soma is obtained.

(See page 156 for this expression) (16)

where $\lambda = +\sqrt{\rho^2-4(\Gamma-\alpha)}$ as in Eq. 6.

For a uniform synaptic resistance change over the whole neuron ($\Gamma = \alpha$; $V_s^* = V^*$), Eq. (16) collapses into the expected simple exponential

$$V(0,\tfrac{t}{\tau}) = V^*(1-e^{-\alpha\frac{t}{\tau}}) \qquad (17)$$

for $\Gamma \neq \alpha$, but at $t/\tau = \infty$ Eq. (16) becomes

$$V(0,\infty) = \frac{V^*\rho\sqrt{\alpha} + V_s^*\Gamma}{\rho\sqrt{\alpha}+\Gamma} \qquad (18)$$

To obtain numerical results from the model a value for ρ must be selected. Eccles (1957, 1961) "standard" cat motoneuron has a value of ρ of 2.3, but, as Rall (1959, 1960) has pointed out, this value is probably too small. Considering that the cell body of the frog motoneuron is smaller than that of the cat motoneuron and that the dendritic extent in the frog is greater than

Eq. 16

$$V(0,\tfrac{t}{\tau}) = \frac{V^{*}\alpha\rho}{\lambda}\left\{\frac{1}{\left(\frac{\rho-\lambda}{2}\right)^2-\alpha}\left[\frac{\rho-\lambda}{2\sqrt{\alpha}}\,\mathrm{erf}\sqrt{\frac{\alpha t}{\tau}} - 1 + e^{\left[\left(\frac{\rho-\lambda}{2}\right)^2-\alpha\right]\frac{t}{\tau}}\,\mathrm{erfc}\left(\frac{\rho-\lambda}{2}\right)\sqrt{\frac{t}{\tau}}\right]\right.$$

$$\left. - \frac{1}{\left(\frac{\rho+\lambda}{2}\right)^2-\alpha}\left[\frac{\rho+\lambda}{2\sqrt{\alpha}}\,\mathrm{erf}\sqrt{\frac{\alpha t}{\tau}} - 1 + e^{\left[\left(\frac{\rho+\lambda}{2}\right)^2-\alpha\right]\frac{t}{\tau}}\,\mathrm{erfc}\left(\frac{\rho+\lambda}{2}\right)\sqrt{\frac{t}{\tau}}\right]\right\}$$

$$+ \frac{\Gamma V_s^{*}}{\lambda}\left\{\frac{1}{\left(\frac{\rho-\lambda}{2}\right)^2-\alpha}\left[\left(\frac{\rho-\lambda}{2}\right) - \sqrt{\alpha}\,\mathrm{erf}\sqrt{\frac{\alpha t}{\tau}} - \left(\frac{\rho-\lambda}{2}\right)e^{\left[\left(\frac{\rho-\lambda}{2}\right)^2-\alpha\right]\frac{t}{\tau}}\,\mathrm{erfc}\left(\frac{\rho-\lambda}{2}\right)\sqrt{\frac{t}{\tau}}\right]\right.$$

$$\left. - \frac{1}{\left(\frac{\rho+\lambda}{2}\right)^2-\alpha}\left[\frac{\rho+\lambda}{2} - \sqrt{\alpha}\,\mathrm{erf}\sqrt{\frac{\alpha t}{\tau}} - \left(\frac{\rho+\lambda}{2}\right)e^{\left[\left(\frac{\rho+\lambda}{2}\right)^2-\alpha\right]\frac{t}{\tau}}\,\mathrm{erfc}\left(\frac{\rho+\lambda}{2}\right)\sqrt{\frac{t}{\tau}}\right]\right\}$$

in the cat, the value of ρ for the frog motoneuron should be larger than that for the cat. A value for ρ of 5 was taken for the present computations. This choice also facilitates comparison with the recent calculations of Rall et al. (1967) in which ρ was usually about 5. Calculations with the present model were mostly carried out at the single time, $t/\tau = .3$ which, with a τ = 4-5ms (Araki and Otani, 1955) would conservatively represent the time of the peak of an actual resistance transient producing a dendritic EPSP with a rise time of 2-3 msec. E_r was taken as the usual -70 mV. The results of the calculations are presented in Table 1. Of prime interest are the cases where the simulated EPSP has a magnitude of about 2.5 mV at $t/\tau = .3$, since the actual dendritic EPSP's were of about this magnitude or smaller. For this size EPSP, produced by only dendritic activity, $\alpha = 1.21$ and the corresponding percent decrease in the height of the voltage response at $t/\tau = .3$ is only about 1%. This shunting reflects only the effect of the changed membrane resistance since the effect of the current injection on the EPSP has been mathematically excluded. Assuming that the rising phase of the M spike can be regarded as representing the response to a naturally-produced current step, then a 1% decrease in peak height is only a 20-40 μV change. And this change is buried in at least 200 μV p-p noise.

To appraise how accurately the calculations based upon the model pertain to the frog motoneuron, one should be aware of two of the more prominent differences between the model and the actual situation. First, in the model the "synaptic activity" is distributed uniformly over the entire dendritic membrane, while in the actual motoneuron the synaptic activity is probably confined to the distal reaches of the dendritic tree. Although the actual value of α in these peripheral locations must be greater than that used in the model if the size of the EPSP is to be preserved, the overall effect

will be a decrease in detectability, since the detrimental effect of displacing the synaptic locus further away from the soma will be dominant during the brief time period of the actual resistance transient. Second, the current step test signal used in the model contains frequencies higher than those present in the actual test signal (the M spike). Since these higher frequencies are the least sensitive to distal resistance changes, the calculations from one aspect underestimate the detectability (% decrease). The above two differences between model and actuality are operating in opposite directions on the detectability measure. While admittedly their opposing effects are not precisely weighed, it is believed that the calculations represent a fair indication of the detectability in the actual situation. Thus, although the model can be taken as only an approximate indicator, it makes it appear quite likely that peripheral dendritic resistance transients will escape detection by present techniques which provide access to the neuron only through the cell body.

TABLE 1.

Location of Synaptic Activity	α	Γ	EPSP Magnitude (mV)	Magnitude of Voltage Response to Current Step (Units of $I_o R_s$)	Percent Change
no synaptic activity	1.0	1.0	0 for all t/τ	0.0814 at $t/\tau = .3$ 0.0544 at $t/\tau = .15$	
dendritic only	2.25	1.0	7 at $t/\tau = .15$	0.053 at $t/\tau = .15$	2.6
dendritic only	2.25	1.0	13.8 at $t/\tau = .3$	0.076 at $t/\tau = .3$	6.6
dendritic only	1.21	1.0	2.56 at $t/\tau = .3$	0.0804 at $t/\tau = .3$	1.2
soma only	1.0	1.46	2.54 at $t/\tau = .3$	0.0789 at $t/\tau = .3$	3.1
soma only	1.0	1.34	1.90 at $t/\tau = .3$	0.0796 at $t/\tau = .3$	2.2
soma only	1.0	1.21	1.18 at $t/\tau = .3$	0.0804 at $t/\tau = .3$	1.2
uniform	1.15	1.15	2.66 at $t/\tau = .3$	0.0796 at $t/\tau = .3$	2.2
uniform	2.25	2.25	19.2 at $t/\tau = .3$		

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BIOGRAPHICAL NOTE

John Simpson attended Rensselaer Polytechnic Institute from 1957 to 1961 and received the degree of Bachelor of Mechanical Engineering in 1961. He was graduated first in the department. He entered the Massachusetts Institute of Technology, Department of Meachnical Engineering in 1961. As a Research Assistant, he was introduced to the irreversible thermodynamics of membranes; this began his interest in physiology. In 1961-1962 he was the recipient of a Sloan Fellowship, and in 1962-1963 as a Teaching Assistant he taught the laboratory portion of Experimental Stress Analysis. He wrote a thesis, "Muscles and Kinematics of the Human Iris," under the supervision of Professor S. Collins and Dr. L. Stark and was awarded the S.M. degree in 1963. In 1964 he was awarded the degree of Mechanical Engineer.

In 1964-1965, Mr. Simpson received a Churchill Fellowship and attended Churchill College, University of Cambridge, where he was introduced to neurophysiological research by Professor Sir Bryan Matthews, chairman of the Physiology Department. In 1965 Simpson returned to M.I.T., and since 1966 he has done neurophysiological research under the supervision of Professors J. Y. Lettvin, T. Sheridan, and P. D. Wall, and Dr. K. Kornacker. From 1965 to 1967 Simpson was awarded a Graduate Fellowship from the National Science Foundation. Since 1968 he has been the recipient of a Training Grant from the National Institutes of Health.

In February, 1969, John Simpson married the former Diane E. Lear.