THE EFFECTS OF RHYTHMIC, LOW FREQUENCY CORTICAL ACTIVATION ON THE EXCITABILITY OF SPINAL CORD MOTOR NEURONS

by

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INTRODUCTION

In the following pages the term cortico-spinal will be used to indicate those neurons with descending axons which traverse longitudinally the medullary pyramids. Since antiquity, men have pondered and attempted to experimentally delineate the function of the tract.

The medullary pyramids are large and must have impressed a number of early anatomists, but no physiological or anatomical significance had been ascribed to these structures until Misticelli in 1709 (33) and Petit in 1710 (37) discovered the decussation of the pyramids. This demonstration that the pyramids crossed appeared to explain the phenomenon first described by Hippocrates that disease of one side of the brain produces motor defects on the contralateral side of the body. Petit was the first man to call the tract motor and felt that the brain subserved the body through cortico-spinal tracts. Thus, the cortico-spinal tract first became generally regarded as subserving motor mechanisms on the basis of inference from limited clinical observations. So deeply was the motor concept of the pyramidal tract ingrained, that it was 190 years before Starlinger (44) thought it worth while to prove the hypothesis to be true by experimental observations. Following section of one or both pyramids in dogs, he observed motor deficits of the contralateral extremities thus furnishing the rational basis for regarding the pyramids as the pathway mediating voluntary control of movements.

In recent years, evidence has accumulated which would indicate

that impulses in the cortico-spinal tract are not the only means by which movement may be initiated (8, 16, 32, 46, 47). At the same time, the importance of anatomically less well defined cortico-fugal pathways has become more apparent (8, 17, 46). Nevertheless, the cortico-spinal tract remains the only anatomically direct pathway from the cerebral cortex to lower motor centers of the cord and appears to subserve an important role in regulating the excitability of spinal cord motor neurons. An understanding of the physiological mechanisms involved, depends in part on an understanding of the cortico-spinal termination in the spinal cord.

Spinal terminations of the pyramidal tract.

After the discovery of pyramidal decussation, attempts were made to trace the extent of the tract and establish its termination. By careful dissection Petit (33) and Mistecilli (37) traced the tract into lateral columns of the upper cervical cord; but due to the limited techniques available, they were unable to follow the fibers further. Türck, in 1851, using the newly discovered principle of Wallerian degeneration as an experimental technique, successfully traced cortico-spinal fibers as far as the lumbar cord but could not delineate any termination in the spinal grey (25). Schafer (40, 41), using Weigert and carmine staining techniques, traced degenerating cortico-spinal fibers, following hemisection of the cord, into the grey matter of the cord. He noted numerous terminations in the small cells at the base of the dorsal horn but was unable to demonstrate terminations in the region of ventral horn cells. In addition, cortico-spinal endings appeared to

be confined to the cervical and lumbar regions of the cord. Schafer's findings were disturbing in light of prevalent anatomical opinion concerning cortico-spinal terminations. Most anatomists and neurophysiologists felt cortico-spinal fibers terminated directly on motor neurons and thus directly influenced their excitability.

That few, if any, cortico-spinal axons do terminate directly on spinal cord motor neurons, has been demonstrated repeatedly in more recent years in a variety of laboratory animals using different histological techniques. Hoff (18) studied the spinal cords of cats using a bouton degeneration technique following lesion of the motor cortex. Cortico-spinal endings were present in relation to cells of the external basilar region of the cord and in relation to internuncials of the central grey. No terminations were found in relation to motor neurons. More recent studies employing the Nauta and Marchi techniques have verified these findings in the cat (9).

In primates (monkeys, chimpanzees, and man), studies using all of the above techniques have demonstrated that a limited number of cortico-spinal fibers do impinge directly on ventral horn cells (19, 22). It should be emphasized that a majority of cortico-spinal terminations in these species occur in relation to internuncials of the cord. Lassek believes the presence of direct cortico-spinal motor neuron connections is related to the ascendency of the species on the phylogenetic scale (24, 25). If this is true and if it is assumed man has reached the highest level on the phylogenetic scale, it may be assumed man should have the highest percentage of direct cortico-spinal connections on motor neurons. The above hypothesis has not been proved.

Other terminations.

This discussion has dealt only with cortico-spinal terminations in the cord. In addition, fibers from the pyramids terminate in the nuclei of the pons and overlying reticular formation (39, 42). Numerous collaterals of cortico-spinal fibers terminate in the bulbar reticular formation (42). Since the reticular formation plays a major role in the excitability of spinal motor centers (2, 21, 26, 31), collaterals of cortico-spinal fibers ending in this region may constitute a secondary pathway for influencing motor mechanisms of the cord. This problem will be discussed later.

Studies of Function.

Physiological studies appear to verify the anatomical patterns of cortico-spinal terminations. Using cats, Lloyd (27) studied activity of cells in the lumbar cord following electrical stimulation of the bulbar pyramids. After a delay of 4-5 msec., following a single stimulus to the bulbar pyramid, postsynaptic cell responses were recorded in the external basilar region of the lumbar cord. In part because of temporal dispersion, single pyramidal shocks were capable of eliciting a prolonged repetitious discharge in these elements lasting 20-30 msec. Repetitive pyramidal stimulation also activated elements in two other regions of the grey matter of the cord: large solitary cells of the dorsal horn; and cells of the intermediate region of the cord, the intermediate nucleus of Cajal. Single pyramidal shocks were incapable of exciting activity in either of the latter two regions. Pyramidal stimulation was incapable of discharging motor neurons. Functional studies

on cats verifies the histological evidence that cortico-spinal fibers terminate exclusively in relation to internuncials of the cord.

Physiological evidence for the presence of direct cortico-spinal motor neuron connections is not nearly so clear. No study comparable to Lloyd's has been performed on primates; however, a few studies are available which support the observation of direct cortico-spinal connection with motor neurons in this group of animals. Bernhard et al (3) and Preston and Whitlock (38) have demonstrated monosynaptic reflex facilitation following single cortical stimuli with a latency too short to permit internuncial delay. Direct excitation of motor neurons, however, occurred only after prolonged tetanic cortical stimulation (3).

Summary

From the anatomical and physiological evidence presented above, the following pattern of cortico-spinal terminations appears to be present: 1) in carnivores (cats), and probably in other "lower" mammals, cortico-spinal fibers terminate exclusively in relation to internuncial cells of the cord; 2) in primates, a limited number of cortico-spinal fibers terminate directly on motor neurons of the cord.

Physiology of the Cortico-Spinal Tract

The spinal terminations of cortico-spinal fibers have been shown both anatomically and physiologically to occur primarily in relation to interneurons. It follows then that cortico-spinal influence on motor neuronal excitability must be accomplished by

influencing this complex system of cord interneurons. Sherrington, in 1906, felt this was the case. Because of Schafers anatomical studies, and through his own observations concerning cortically induced movements, Sherrington felt the cortico-spinal system was an internuncial path. "As such it is concerned with movements - to which any anatomical or directional attributes are secondary." (43).

To avoid inadvertent activation of descending pathways other than the cortico-spinal tract, Lloyd (27) chose to study the spinal effects of cortico-spinal activity after stimulation of anatomically isolated medullary pyramids. As has been stated previously, he was unable to cause movements by stimulation of isolated pyramids or record synchronous discharge from ventral roots. However, he observed that pyramidal stimulation did influence evoked monosegmental reflex discharges. Short trains of three or more pyramidal shocks resulted in facilitation of both monosynaptic and polysynaptic reflex arcs. Polysynaptic facilitation was observed to have a latency of 9-12 msec. following the first pyramidal shock, whereas facilitation of the monosynaptic reflex activity appeared after an additional delay of 2-3 msec. Facilitatory effects persisted for 30-40 msec. following the cessation of pyramidal stimulation.

Brookhart (6) and Landau (23) have caused movements by high frequency trains of stimulation to unisolated medullary pyramids in cats. The pattern of movements observed were quite irregular and unpredictable. Landau observed that the parameters of medullary pyramidal stimulation necessary to cause movements were quite variable from preparation to preparation and could vary considerably in the same preparation from

time to time. Patton and Amassian (36) state that it is difficult to arrive at any conclusions from this type of study. In their opinion, the possibility of spread of stimulus to surrounding structures such as the medial lemniscus or reticular formation was not adequately controlled. Movements observed may have resulted from excitation of these structures in addition to excitation of the cortico-spinal tract (36).

In cats, a species in which cortico-spinal fibers terminate exclusively on internuncials, a single volley of impulses confined to cortico-spinal fibers will not result in motor neuron excitation. It may be concluded that the facilitatory action of such impulses on both the monosynaptic and polysynaptic reflexes is mediated through internuncial pathways. No inhibitory effects have been reported in this species.

It has already been pointed out that direct connections exist between cortico-spinal fibers and some motor neurons in monkeys. The limited number of studies on this form permit the conclusion that monosynaptically mediated facilitation of some motor neurons does occur. The possibility that inhibitory effects may also be exerted by cortico-spinal fibers has only recently been supported by experimental evidence. Preston and Whitlock (38) published reports from a rather extensive study on the spinal effects of motor cortex stimulation in monkeys with large lesions of the mesencephalic reticular formation. This brain lesion interrupted all descending tracts above the lesion with the exception of the cortico-spinal and cortico-bulbar tracts. These investigators noted a rather stereotyped triphasic influence of single cortico-spinal volleys on monosynaptic reflexes. After a brief, initial

period of facilitation, there occurred inhibition of the monosynaptic reflex, followed by a later period of facilitation which persisted for about 20 msec. Small doses of nembutal blocked the observed inhibition without altering the facilitation. Intra-cellular recording showed that cortical or pyramidal stimuli generated only excitatory post synaptic potentials (epsp's) in some motor neurons. In other motor neurons, cortical or pyramidal stimulation generated only inhibitory post synaptic potentials (ipsp's) with a latency consistent with the latency for inhibition of the reflex. Stimulation generated in other neurons both epsp's and ipsp's. This evidence led Preston and Whitlock to the conclusion that different cortico-spinal fibers may evoke either facilitatory or inhibitory influences on spinal cord motor neurons. Since the electrical stimulation initiated activity in both types of fibers simultaneously, it is understandable that both facilitatory and inhibitory influences were observed at different times after the cortical or pyramidal shock. Because of the latent periods involved, and because the inhibition could be blocked by anesthetics, Preston and Whitlock concluded that the inhibitory and late facilitatory influences were mediated over pathways involving internuncial neurons.

Brookhart's study (6) involving stimulation of unisolated pyramids in monkeys has been subjected to the same criticism as has the similar study of Landau (23) in cats (see above). However, the study appears to shed some light on the functional significance of direct cortico-spinal motor neuron terminations. A careful analysis of the perameters of pyramidal stimulation needed to induce movements led Brookhart to suggest the following hypothesis: 1) large, low threshhold, rapidly

conducting cortico-spinal fibers terminate primarily in relation to motor neurons, and probably are responsible for the phasic element of cortico-spinal control; 2) smaller, higher threshhold, more slowly conducting fibers appear to terminate principally in relation to interneurons. These are probably responsible for the tonic element of cortico-spinal function.

Secondary pathways of pyramidal influence.

All the above hypotheses and observations concerning cortico-spinal function are based on the assumption that cortico-spinal activity reached the cord only by means of cortico-spinal fibers. That cortical influences on motor neurons may be subserved by a secondary, less direct pathway has been suggested by the anatomical findings of cortico-spinal collaterals to the brain stem reticular formation, and by some of the observations made by Preston and Whitlock (38). The regulation of spinal reflexes by reticulospinal influences has been well documented by several studies (2, 21, 26, 31). Preston and Whitlock observed that the latency of the delayed period of facilitation of monosynaptic reflexes following cortical or pyramidal stimulation was comparable to the latency for facilitation of reflex activity following stimulation of the reticular formation. Consequently, they were forced to conclude that this secondary facilitation could have been mediated by cortico-bulbo spinal pathways rather than direct cortico spinal pathways. Furthermore, no evidence was presented which clearly shows that the observed inhibition of monosynaptic reflexes was mediated only by cortico-spinal fibers.

Summary

The function of the pyramidal system can be summarized as follows: 1) the cortico-spinal system influences motor performance by regulating or modulating spinal reflexes through its influence on the excitability of interneurons. Cortico-spinal fibers may subserve an inhibitory influence on reflex patterns; however, this phase of cortico-spinal action is still poorly understood. 2) Monosynaptic regulation of motor neuron excitability originating from the cerebral cortex appears to be a recent phylogenetic addition and has been noted only in the higher primates. 3) Cortical control may be exerted by less direct pathways through collaterals of cortico-spinal fibers entering into the brain stem reticular formation.

Synaptic Activation of the Cortico-Spinal System.

In all studies presented above, cortico-spinal elements were excited by directly stimulating cortico-spinal neurons or axons. A number of studies indicate other techniques are available for initiation of cortico-spinal activity. Adrian and Moruzzi (1) noted the appearance of bursts of rhythmic cortical activity in the 8-12 cycle per second range in animals under barbiturate anesthesia. Many of these cortical waves were accompanied by the discharge of impulses in cortico-spinal fibers.

A cortical waveform similar to that produced by anesthetics can be recorded from the cerebral cortex of animals with transsection of the brain stem at the level of the mesencephalon (4, 5, 7, 48). Bremer (5) has termed the train of rhythmic 8-12 cycle per second cortical

waves produced in this manner spindles or spindle bursts. Whitlock et al (48) and Brookhart and Zanchetti (7) have shown that some spindle waves excite corticospinal elements. This phenomenon has been studied using a "pyramidal" preparation in which a mesencephalic lesion is carefully placed to spare cortico-spinal fibers in the base of the peduncle. Such a preparation will spindle actively and excite cortico-spinal elements, but the animal will remain quiet (48).

Rhythmic stimulation of the ventro-lateral thalamus, internal capsule or medial lamniscus produces a train of characteristic electrocortical waves, the augmenting response (11, 12, 34). Brookhart and Zanchetti (7) have shown that stimulation of the ventro-lateral thalamus will activate cortico-spinal elements. The bursts of impulses originating at the cortical level can be detected by recording cortico-spinal activity from the medullary pyramids.

Summary.

Thalamic stimulation and spontaneous spindling synaptically drives corticospinal units into rhythmic bursts at a frequency of 8-12 cycles per second (7). Cortico-spinal activity evoked in this manner will not cause movements. Adrian and Moruzzi have postulated that the frequency of cortico-spinal discharge is too low to excite spinal cord motor neurons (1). No effort has been made to test this hypothesis; moreover, no attempt has been made to see whether cortico-spinal activation by these two techniques has any regulatory effect on the excitability of spinal cord motor neurons.

Statement of the Problem

The effects of repetitious cortico-spinal discharge induced in bursts or clusters by rhythmic low frequency stimulation of the thalamus or spindle waves are completely unknown. The present series of experiments was designed to test whether relayed cortico-spinal impulses generated by stimulation of the ventro-lateral thalamus or during spontaneous spindling could regulate motor neuron excitability.

If one assumes that cortico-spinal activity will regulate the excitability of spinal cord motor neurons, it should be possible to test this assumption by initiating spinal reflexes in relation to bursts of cortico-spinal activity. If it is assumed that the magnitude of reflex discharges reflect the level of motor neuron excitability, any observed changes in the magnitudes of reflexes during conditioning would be indicative of changes in the excitability of spinal cord motor neurons as a result of conditioning. Utilizing the same type of experimental procedure, it should be possible to test whether a temporal relationship between the initiation of conditioning activity and the initiation of a reflex is necessary for impulses from the cortico-spinal tract to regulate the excitability of spinal cord motor neurons.

METHODS

Cats were used in all experiments. The data to be discussed have been derived from fourteen animals.

Under ether anesthesia, the trachea was canulated, and both carotid arteries were isolated by means of loose ligatures. The animal was then placed in a stereotaxic frame and the skull was exposed. Trephine holes were placed to permit introduction of electrodes into the mesencephalon, thalamus, and pyramid. The right frontal cortex was exposed through the frontal sinus. In order to avoid interference from anesthetic depression, a large lesion designed to spare only cortico-spinal and cortico-bulbar fibers in the basis pedunculi was then made in the mesencephalic tegmentum at the midcollicular level. Destruction was carried out by passing high frequency electrical current between two stereotaxically placed electrodes four mm. apart and insulated except for four mm. at their tips. The coagulating current was supplied by a Burdick electro-coagulation apparatus. A total of five coagulations was required to produce the desired lesion. Following this procedure ether was discontinued. The animal remained somnolent throughout the remainder of the experimental period.

In those animals in which thalamic stimulation was used as the conditioning procedure, a bipolar concentric electrode with a tip separation of 0.5 mm. was placed stereotaxically in nucleus ventralis posterolateralis.

It was necessary to monitor cortico-spinal activity. One method of

monitoring is simply recording augmenting responses produced by stimulation of the ventro-lateral thalamus, and spontaneous spindle bursts from the motor cortex. This is a reasonable technique when using thalamic stimulation to generate cortico-spinal activity since each thalamic stimulus will invariably evoke a relayed cortico-spinal volley (7). Spindling is much more irregular, and will frequently occur over one side of the brain or over only one portion of the cortex (5). Moreover, the temporal relations between relayed pyramidal volleys and spindle waves recorded from punctate foci on the cortex are not uniform (7, 48). For this reason, cortico-spinal activity was monitored from a small electrode placed in the bulbar pyramid.

The monitoring pyramidal electrode was introduced into the right pyramid at an angle of 30° through the posterior cerebellum. Pyramidal monitoring electrodes were bipolarly concentric with a tip separation of about 2 mm. The core wire was either tungsten or nichrome. The tips of the core wires were tapered by electrolysis, and all electrodes used had tip diameters ranging from 2-6 mu (14, 20). These electrodes had the advantage of being sensitive, small, and rigid. A properly constructed electrode could penetrate easily the multiple pial barriers encountered without distortion of the tip, and could be used several times. Because of their small size, very little pyramidal damage was produced by placing these electrodes in the pyramids. Examination of the tissue histologically afforded confirmation of minimal damage due to electrode penetration. This observation has been tested further. Relayed pyramidal volleys were recorded unchanged in amplitude or waveform from the same electrode reinserted distally in the same pyramid. Furthermore, it was noted

that the level of excitability of spinal motor neurons was altered during spindling. The level of excitability of motor neurons during interspindle lulls was different from that observed during spindling but did not change after section of the pyramids.

In preliminary experiments, an attempt was made to elicit spinal reflexes by stimulating dorsal roots and recording from ventral roots of the same spinal segment. The operative procedures involved with this technique are quite extensive, and because of trauma, shock, and blood loss, the animals subjected to these procedures either did not survive surgery, or were in such poor condition that reflexes were not obtained. This necessitated the employment of compromise techniques for elicitation of reflexes. These were: 1) stimulating and recording from different peripheral nerves (stimulate peroneal nerve, and record from tibial nerve); 2) stimulating and recording from the same mixed peripheral nerve (stimulate sciatic nerve, and record from tibial nerve). The former technique was used in five cats, and had the major disadvantage that no monosynaptic reflexes could be elicited in this manner (28). Since it was imperative to note the effects of cortico-spinal conditioning on both the monosynaptic and polysynaptic components of spinal reflexes, the latter technique was used in the majority of experiments. Stimulation of a mixed nerve introduces an additional variable, that of antidromic depression of motor neurons (29, 30). The degree of antidromic depression should remain relatively constant throughout an experimental period provided the parameters of stimulation remain constant throughout the experimental period (29, 30).

These nerves were approached by incising the skin and fat over the

popliteal space of the left leg. As great a length of tibial nerve as possible was carefully dissected free from surrounding connective tissue. The dissection of the sciatic nerve was carried centrally past the emergence of the nerves to the hamstring muscles and, distally the tibial nerve was dissected until it began to break up into numerous muscular branches to the plantar flexors of the foot. All muscle branches of the sciatic and tibial nerves were severed. Silver wire stimulating electrodes were placed high on the sciatic trunk, and a pair of silver wire recording electrodes placed near the cut end of the tibial nerve. The distance between stimulating and recording electrodes in all experiments was about five centimeters. In those experiments in which the peroneal nerve was used for stimulation, the peroneal nerve was dissected free and placed on silver wire stimulating electrodes.

Stimulating pulses were rectangular pulses led to the preparation through isolation transformers. For thalamic stimulation, one second trains of pulses at a frequency of eight per second were used routinely. Pulses of 0.3-0.6 msec. duration and about five volts intensity were usually sufficient to produce augmenting waves accompanied by relayed pyramidal volleys. Stimulation of the peripheral nerve was induced by single pulses of .01-.04 msec. duration; 1-2 volts intensity usually produced an adequate test response. Activity from the pyramidal electrode and peripheral nerve electrodes was led through preamplifiers and displayed oscillographically. One oscilloscope was used to display the reflex, the other monitored pyramidal activity.

To better evaluate any effects of conditioning on spinal reflexes and to test whether any conditioning effects depended upon a critical

time relationship between the elicitation of a test reflex and thalamic stimulation or spontaneous spindle waves, arrangements were made to measure the time difference between the initiation of a test response and a single thalamic stimulus or spontaneous spindle wave. The nerve stimulus pulse was led through a capacitor to the vertical input of the cathode ray oscilloscope monitoring conditioning activity to produce a visible artifact on the record indicating the time of initiation of the test response. By varying delay functions, it was possible to time the occurrence of the test response in either of two ways. The test response could be initiated at any selected time during the entire train of thalamic shocks or at any time during a burst of spindle waves. An alternative delay function permitted the elicitation of the test response at any instant during a single evoked or spontaneous wave included within the burst. To secure an adequate sample, a routine practice of recording six test reflexes at a single delay setting was adopted. In most experiments, reflexes were tested following the third or fourth relayed volley.

Since the spindling was spontaneous rather than evoked, it was necessary to provide a mechanism utilizing spontaneous activity to trigger a testing reflex. This was accomplished by leading voltage from the vertical deflection plates of the oscilloscope monitoring pyramidal activity to the trigger of a waveform generator. When the voltage on the vertical deflection plates due to a spindle wave reached a critical level, the waveform generator was triggered and produced a negative going sawtooth which triggered the pulse generator providing a stimulus to the peripheral nerve. By carefully varying delay settings along the

triggering sawtooth, it was possible to vary the timing of the reflex in relation to the spindle burst or a single spindle wave.

Permanent records of the oscilloscopically displayed responses were made by photographing the oscilloscope screens. It was found desirable to record the pyramidal response using the excursion of a beam stationary along its time axis and to record on running film. The reflex was displayed on a moving beam and recorded on the same film. Following the processing of the film, a photographic enlarger was used to permit the tracing of the responses onto paper for more accurate measurement. Measurements of amplitudes of monosynaptic reflexes and areas of polysynaptic reflexes were made from the tracings. The monosynaptically initiated reflex discharge represents a highly synchronous discharge of motor neurons. The magnitude of this response is most easily ascertained by a measurement of the amplitude of this discharge. The polysynaptically induced discharge is a highly asynchronous discharge of motor neurons occupying a much longer duration and exhibits a great deal of temporal dispersion. Its magnitude could be best estimated by measuring the total area of the response. This was accomplished by using a planimeter.

At the completion of each experiment, the brain was injected with formalin through a carotid artery and allowed to harden in situ. Following removal and proper fixation in ten percent formalin the brains were blocked, frozen, and sectioned at 150-175 microns. The wet sections were mounted on a glass slide and photographed by means of a photographic enlarger (15). This technique permitted the location of the position of thalamic and pyramidal electrode tips and the verifica-

tion of the extent of mesencephalic coagulation.

Analysis of Data

All experiments were designed to permit thorough statistical analysis of the results obtained. All raw data scores of a single experiment for amplitudes and areas of the populations of unconditioned responses were compared with amplitudes and areas for the populations of conditioned responses by a single classification variable analysis. If the "F" number obtained by this analysis was significant, "t" tests were performed to compare the means obtained (13).

In experiments using thalamic stimulation to provide cortico-spinal conditioning activity, it was possible to replicate six responses at the same time interval following a single thalamic stimulus in a train of eight. To increase the sample size without, at the same time, multiplying the work of measurements of these responses, each group of six replications was superimposed (fig. 1). From the superimposed tracings, midrange values of monosynaptic amplitudes and polysynaptic areas could be obtained. Standard deviation for each group of six superimposed replications was estimated from the range (13). The major disadvantage of this technique was that the only estimate of an arithmetic mean for each group of six responses was the midrange, a non-parametric statistic (13). The estimate of the standard deviation from the range with the size sample used is 93.2 percent efficient (13).

Results obtained from superimposed tracings were analyzed by two techniques. Since mid-range values are not well adapted to standard statistical testing procedures, each mid-range value for a group of six

superimposed responses was compared by means of the sign test to the mean value unconditioned reflex responses obtained in the same experiment. In this test the sign of the difference of a conditioned mid-range value and the mean value of unconditioned responses was recorded. This procedure was repeated to compare all mid-range values obtained from a single experiment with the mean of unconditioned responses for that experiment. The number of the less frequent sign is designated "r". The probable significance of the differences could then be estimated within specified limits through the use of tables of "r" values.

In order more rigorously to determine whether a critical factor of time between the initiation of a conditioning volley and the initiation of a reflex existed, the standard deviations of the data from each animal at a sequence of time intervals were subjected to analysis of variance. This procedure tested whether the scatter of excitabilities could be affected by the time interval between conditioning and testing. To assist the analysis of variance, the time following a thalamic shock was divided into ten msec. intervals. The standard deviations of all responses occurring in each ten msec. interval were grouped together. Standard deviations for all groups of responses occurring within 0-10 msec. of the initiation of a single thalamic stimulus formed one category. Those from 11-20 msec., another category, and so on. If a significant "F" number was obtained, the mean standard deviation for each category was compared to all other categories by "t" tests.

In animals in which spontaneous spindles provided cortico-spinal conditioning activity, it proved impossible to initiate six reflexes with the identical relationships to relayed spindle volley. For this

reason these responses were not superimposed, but were compared with a single classification analysis as described above. The amplitudes and areas of conditioned responses were grouped in the same manner as described above and subjected to analysis of variance.

All analyses compared conditioned and unconditioned responses in single experiments. Because of the tremendous range of amplitudes, areas, and variances of unconditioned responses between cats, no attempt was made to analyse data between cats. Furthermore, since the changes in the individual components of the conditioned test responses were inconstant from cat to cat, any possibility of group analysis was precluded.

RESULTS

Conditioning.

Cortico-spinal conditioning activity, whether generated by thalamic stimulation or spontaneous spindling, was monitored by electrodes placed in the medullary pyramids of all cats.

A relayed volley produced by electrical stimulation of nucleus ventralis posterolateralis arrived at the pyramidal electrode after a delay of about ten msec. The fourth and fifth of a sequence of eight relayed volleys evoked by thalamic stimulation are illustrated at the bottom of figure 1. The predominant positivity of the evoked response is presumed to be the result of recording from killed ends of pyramidal fibers (35). A second artifact is seen after the fourth response. This represents the artifact imposed on the record from the pulse generator providing the peripheral nerve stimulus. It was presumed that the measured interval between thalamic stimulus and the nerve stimulus was the time interval between the initiation of the two responses. The upper portion of figure 1 illustrates six reflex discharges which were initiated at the interval of time indicated on the pyramidal recording. These reflex discharges were traced in the superimposed fashion indicated. A further description of the reflex will follow.

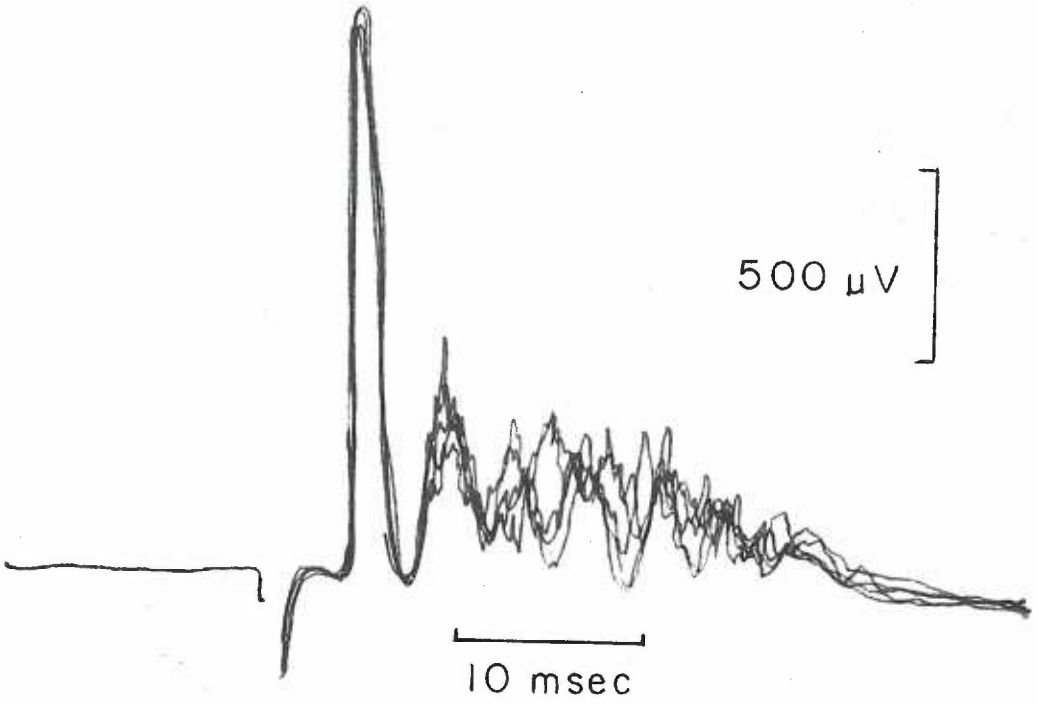
The first four relayed volleys generated during a spindle burst are illustrated in figure 2. Note that the predominant waveform of this type of relayed activity is also positive. The artifact which distorts the third relayed volley was again taken at the time at which the peripheral

Figure 1

CHARACTERISTIC EXAMPLES OF REFLEXES AND RELAYED CORTICO-SPINAL VOLLEYS DURING A TRAIN OF AUGMENTING WAVES EVOKED BY THALAMIC STIMULATION.

The upper portion of this figure illustrates six reflex discharges recorded from the tibial nerve following stimulation of the tibial nerve. For each response, the stimulation of the nerve occurred at the same interval following the fourth thalamic stimulus in a train of eight. The gap in the recording that occurs immediately after the stimulus artifact represents the action potential transmitted distad as a result of direct excitation of fibers of the tibial nerve. The sharp negative wave beginning five msec. after the stimulus artifact is the result of monosynaptic activation of motor neurons of the spinal cord. The latter group of negative waves is the polysynaptic reflex.

The lower portion of the figure illustrates the fourth and fifth relayed volleys of a train of eight relayed volleys evoked by stimulation of nucleus ventralis posterolateralis. The recording electrode was in the ipsilateral medullary pyramid. The artifact following the fourth relayed volley is the artifact imposed on the record from the pulse generator supplying the stimulus to the tibial nerve in order to evoke the reflex discharge. Time and voltage calibrations as indicated.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10 msec

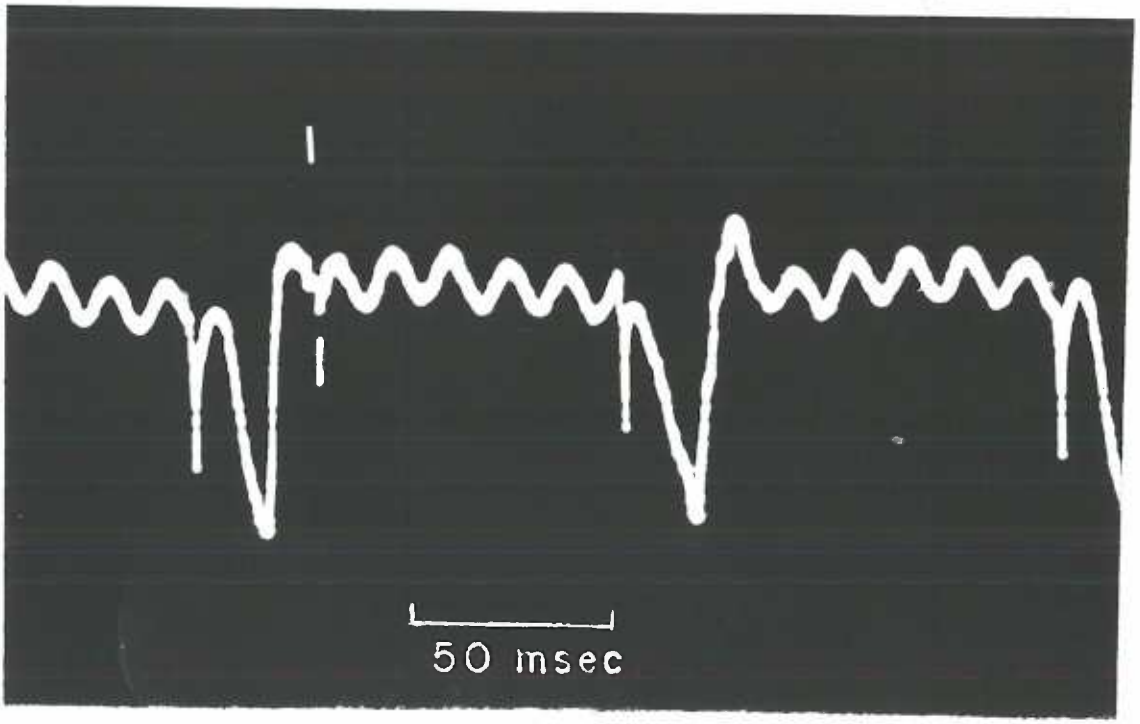
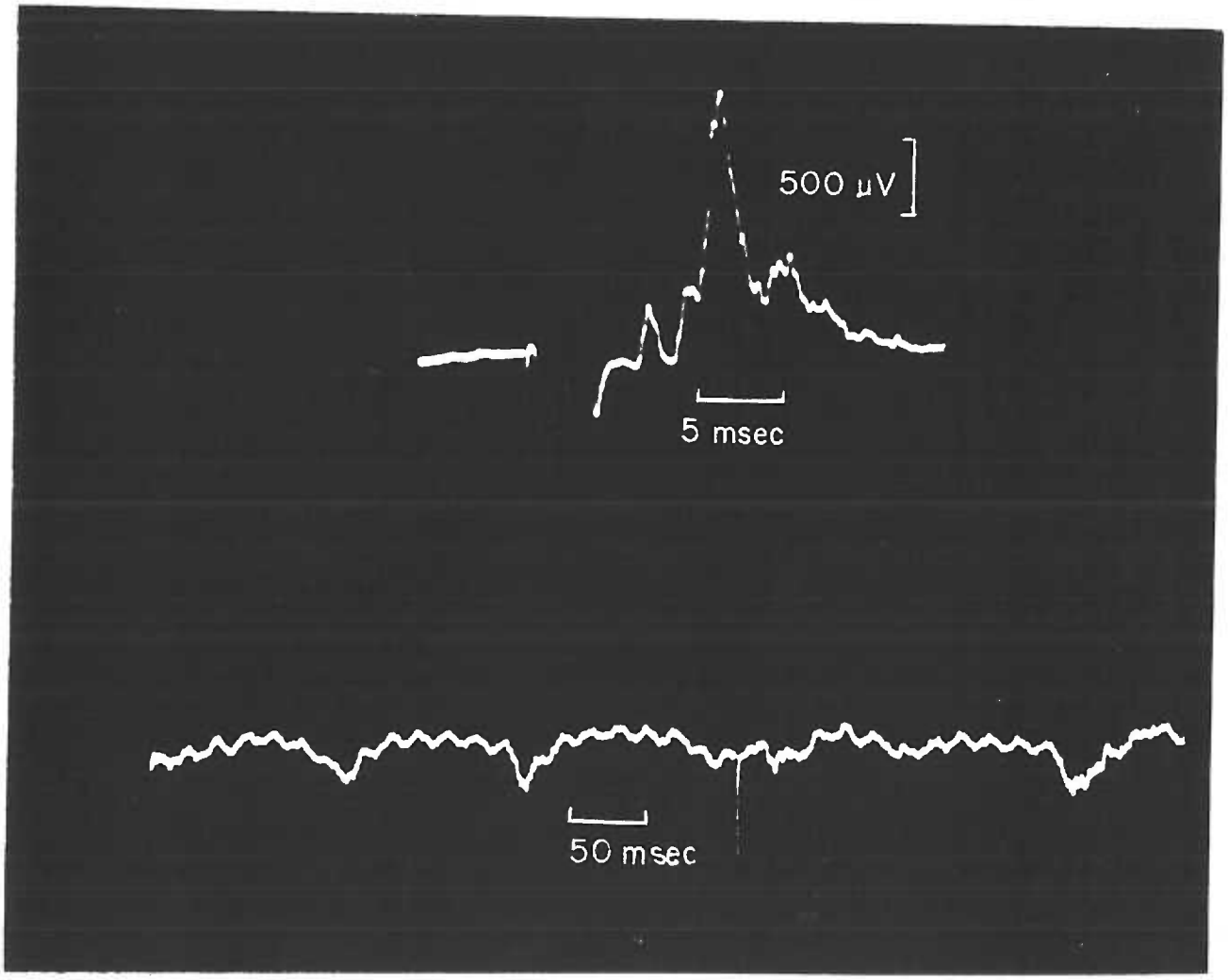

50 msec

Figure 2

TYPICAL EXAMPLES OF A REFLEX AND RELAYED CORTICO-SPINAL VOLLEYS ACCOMPANYING SPINDLING.

The upper figure is a reflex discharge evoked by tibial nerve stimulation. Note again the characteristic features of the reflex response. The lower figure illustrates the first four pyramidal volleys accompanying a spontaneous spindle burst. The artifact which distorts the third volley is the stimulus artifact imposed on the record to denote the instant of peripheral nerve stimulation. This example is typical of the pyramidal activity recorded from a spindling cat.


500 µV
5 msec
50 msec

nerve was stimulated. The interval between the onset of positivity of a given relayed volley and the artifact is taken to be the time interval between conditioning and testing. The upper portion of the figure is a photograph of the reflex discharge initiated at the time interval indicated on the lower tracing.

Testing.

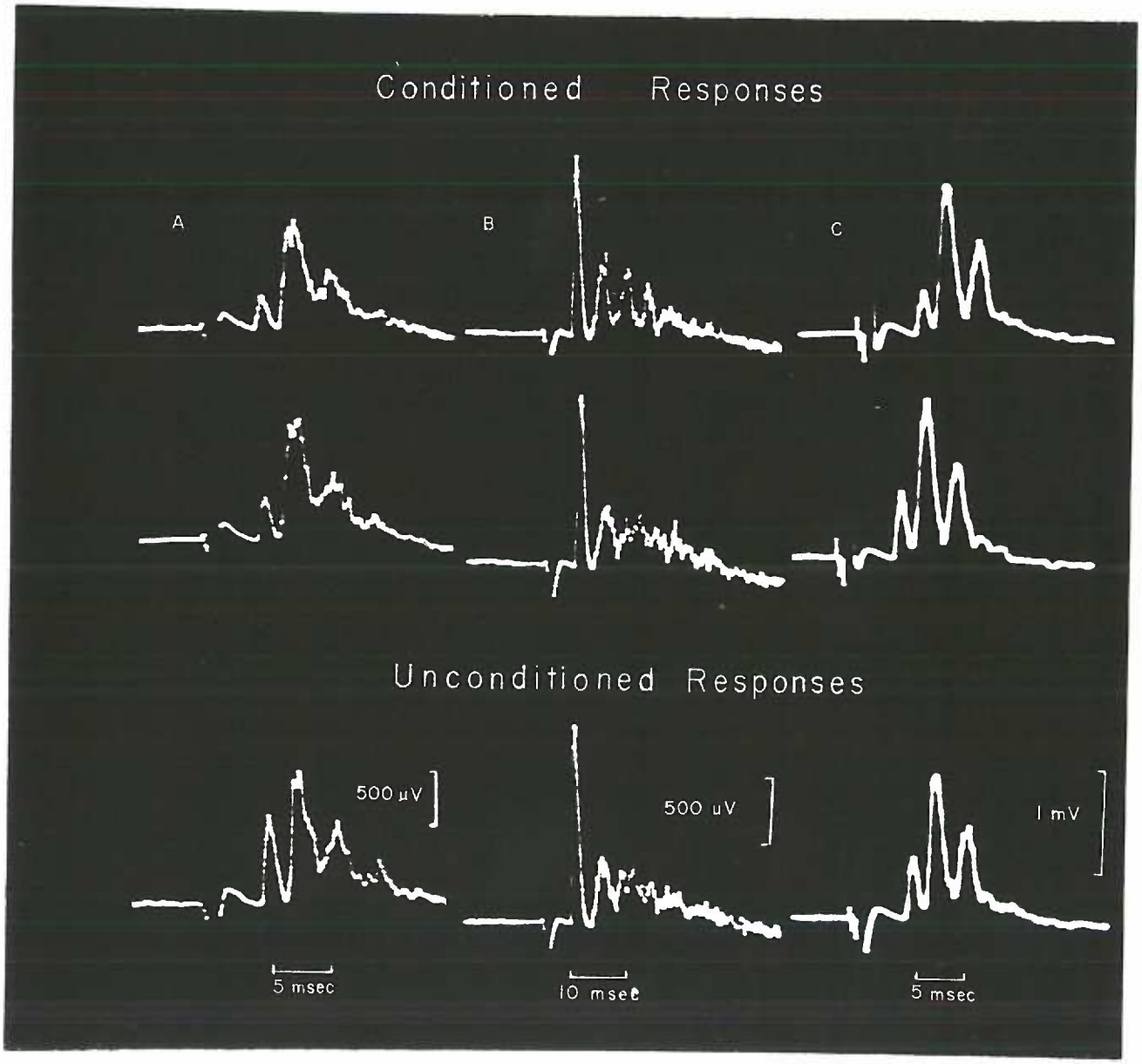
Reflex responses obtained by stimulating and recording from the same peripheral nerve were similar in waveform to those obtained by Lloyd (28). Examples of this complex response are seen in figure 3. The initial portion of the recording, following immediately after the stimulus artifact, is actually a gap in the tracing. This gap represents the interval occupied by the action potential transmitted distal as a result of direct stimulation of the fibers of the tibial nerve. The action potential does not appear on the photograph because of its rapid time course and high amplitude. The sharp negative wave beginning 5-6.5 msec. after the stimulus artifact is produced by highly synchronous impulses evoked by monosynaptic activation of motor neurons in the spinal cord. The latter portion of each reflex, made up of a complex series of negative waves, represents the polysynaptically induced reflex discharge. Because of the duration and high degree of temporal dispersion of the polysynaptically induced discharge, its magnitude could be best estimated by measuring its total area.

All testing reflexes were elicited by suprathreshhold but not maximal stimulation. Parameters of stimulation necessary to elicit these reflexes were similar in all cats. Single pulses of 1-3 volts intensity

Figure 3

EXAMPLES OF CONDITIONED AND UNCONDITIONED REFLEXES OBTAINED BY STIMULATING AND RECORDING FROM THE TIBIAL NERVE.

The conditioned reflexes were elicited in relation to thalamically evoked relayed volleys which were recorded from the medullary pyramid. Examples are presented from three separate experiments represented by columns A, B, and C. Note the variability in the magnitude of the individual components of the reflexes obtained in different experiments. Time and voltage calibration are indicated at the bottom of each column. Description of the reflexes is in the text.


Conditioned Responses
A
B
C
Unconditioned Responses
500 µV
500 uV
1 mV
5 msec
10 msec
5 msec

and 0.01-0.04 msec. duration were usually sufficient to elicit an adequate testing reflex.

Analysis of Results.

A description of the statistical tools used to analyze the data has been described above. The amplitudes and areas of contitioned responses were compared to the amplitudes and areas of unconditioned responses using single unit variable analyses, "t" tests, and sign tests. The mean level of excitability of the motor neuron pools tested would be reflected by significant alterations in these measurements.

The results from all experiments are summarized in Table I. Column "I" of this table is a statement of the changes in amplitudes and areas of all conditioned reflex discharges compared to all unconditioned reflexes in each experiment. The reflexes were either initiated at unselected times during the entire train of conditioning activity or at selected times during a short segment of the train. The reflexes tested in Experiments 1-3 were elicited randomly over an entire train of eight thalamic stimuli. All the reflexes obtained from spindling cats were obtained in a similar random relationship to spindle bursts (Exp. 11-14). In Experiments 4-10, the reflexes tested were elicited during the period of a single volley in a train of eight thalamically evoked relayed volleys. Reflexes were initiated during and following the second relayed volley in Experiment 7; during and following the third volley in Experiments 4, 5, and 8; during and following the fourth volley in Experiments 6 and 10; and during and following the fifth volley in Experiment 9. Column "H" of Table I lists the "t" score or "r" value

TABLE I

MONOSYNAPTIC REFLEX

	A	B	C	D	E	F	G	H	I
Exp. No.	Cond. mean	Cond. S^2	Cond. S	Uncond. mean	Uncond. S^2	Uncond. S	F test	T test or Sign test	A - D Change
6			115.03	510.5	2873	53.6	2.39	r(+) = 7	
7			30.94	213.8	4147	64.4	1.91	r(-) = 2	
8			29.4	227.6	718.2	26.8	2.22	r(+) = 4	decr.
9			71.76	874.5	6037	77.7	0.056	r(+) = 3	decr.
10			51.87	1564.4	2030	53.2	1.75	r(+) = 4	decr.
11*	351.88	3588	59.9	305	949.87	30.82	8.17	2.86	incr.
12*	121.3	750.8	27.4	157.1	1004.7	31.7	8.67	2.94	decr.
13*	472.4	2134	46.3	626.8	1673.2	40.9	10.06	3.17	incr.
14*	711.4	3745.4	61.2	857.2	2313.6	48.1	21385	15.4	decr.

* The asterisk denotes those experiments employing spindling as the conditioning procedure. The units for columns A & D of the monosynaptic table are μV. The units for columns A&D of the polysynaptic table are mV·msec.

T A B L E I (cont.)

POLYSYNAPTIC REFLEX

Exp. No.	A Cond. mean	B Cond. S2	C Cond. S	D Uncond. mean	E Uncond. S2	F Uncond. S	G F test	H T test or Sign test	I Change A - D
1	3.47	0.109	0.138	1.23	0.015	0.122	454.85	20.13	incr.
2	2.68	0.477	0.218	3.53	0.596	0.772	21.13	4.59	decr.
3	1.60	0.089	0.298	1.26	0.057	0.239	12.62	3.55	incr.
4			0.337	1.15	0.167	0.409	0.20	r(-) = 0	incr.
5			0.181	0.330	0.064	0.079	2.33	r(-) = 3	incr.
6			0.538	3.25	0.133	0.365	1	r(+) = 0	decr.
7			0.492	5.11	0.407	0.638	3.33	r(+) = 0	decr.
8			0.341	4.33	0.105	0.324	1.28	r(-) = 0	incr.
9			0.592	5.52	0.198	0.445	0.056	r(+) = 0	decr.
10			1.39	5.19	1.69	1.30	0.567	r(+) = 7	ins.
11*	2.16	0.305	0.552	0.960	0.128	0.358	9.16	3.04	incr.
12*	5.60	0.199	0.446	6.58	0.321	0.567	19.91	4.46	decr.
13*	3.04	0.311	0.558	2.78	0.097	0.311	2.21		ins.
14*	8.79	0.960	0.980	9.70	0.593	0.770	1.76	4.53	decr.

obtained when comparing the conditioned and unconditioned amplitudes and areas for each experiment. All these values but three are significant at the 95 percent confidence level.

Monosynaptic reflexes evoked during relayed pyramidal volleys produced by thalamic stimulation were utilized in five cats. The monosynaptic reflex was facilitated in one of these cats (Exp. 7). This observation indicates that thalamic stimulation raised the level of excitability of the motor neuron pools tested in this animal. The monosynaptic reflex was depressed by relayed discharges evoked by thalamic stimulation in three other cats (Exp. 8, 9, and 10). Graphic illustrations of the results obtained from two of these preparations (Exp. 8 and 9) are presented on graphs "A" of figures 4 and 5. Significant depression of the monosynaptic reflex in these preparations indicates that relayed cortico-spinal volleys evoked by thalamic stimulation resulted in a decrease in the level of excitability of the motor neurons tested in these animals.

This same type of variable reaction of the monosynaptic reflex to conditioning existed when relayed cortico-spinal volleys accompanying spontaneous spindling were used as the conditioning procedure. The monosynaptic reflex was facilitated in two spindling animals (Exp. 11 and 13) and depressed in two other animals (Exp. 12 and 14). The results from Experiment 12 are illustrated in graph "A" of figure 6.

Polysynaptic reflexes were either facilitated or depressed during cortico-spinal conditioning activity. Cortico-spinal volleys evoked by thalamic stimulation facilitated the polysynaptic reflex in five cats (Exp. 1, 3, 4, 5, and 8). Results from Experiment 8 are illustrated in

Figure 4

GRAPHIC ILLUSTRATION OF THE CHANGES IN THE MAGNITUDE OF THE MONOSYNAPTIC AND POLYSYNAPTIC REFLEX DISCHARGES AS A RESULT OF THALAMIC STIMULATION.

Graph "A" illustrates the changes in amplitude of the monosynaptic reflex before, during, and after the third volley in a train of eight thalamically evoked relayed volleys. Graph "B" illustrates the changes in area of the polysynaptic reflex. The initiation of the thalamic stimulus is at time zero. The relayed volley was recorded from the level of the medulla about ten msec. later and persisted for 20-25 msec. In each graph the solid line represents the mean value of unconditioned reflexes. The two dotted lines express two standard deviations above and below the mean. Each plotted point represents the mid-range of six superimposed responses tested at the time intervals indicated. The length of the vertical line through each point denotes two standard deviations on either side of the mid-range. In this particular experiment, the monosynaptic reflex discharges were depressed during the interval occupied by the train of conditioning volleys. The polysynaptic reflex was facilitated during the interval occupied by the train of conditioning volleys. The amplitude scale of graph "A" should read μV.

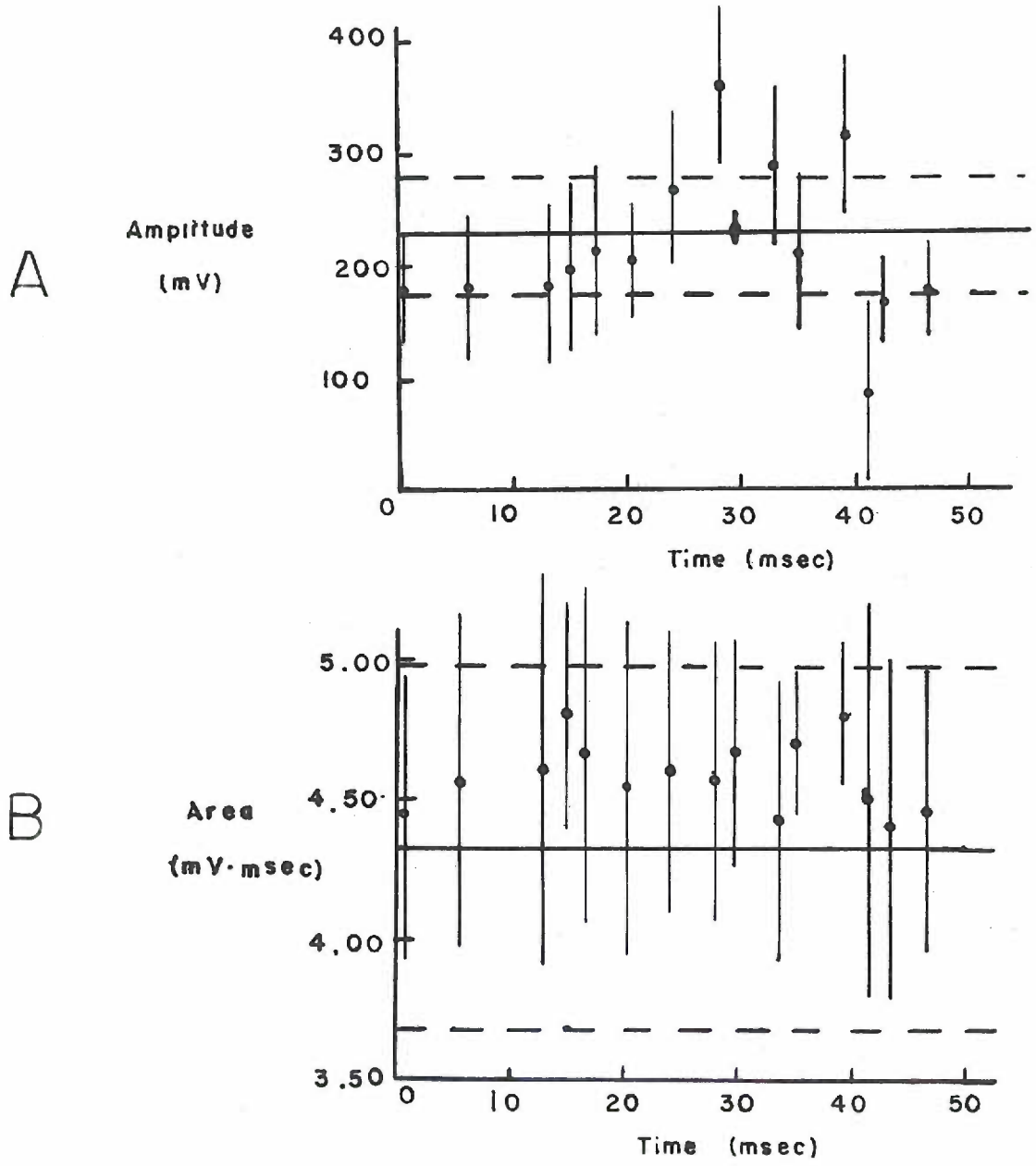

A
Amplitude
(mV)
400
300
200
100
0
10
20
30
40
50
Time (msec)
B
Area
(mV·msec)
5.00
4.50
4.00
3.50
0
10
20
30
40
50
Time (msec)

Figure 5

GRAPHIC ILLUSTRATION OF THE CHANGES IN THE MAGNITUDE OF THE MONOSYNAPTIC AND POLYSYNAPTIC REFLEX DISCHARGES AS A RESULT OF THALAMIC STIMULATION.

The graphs are identical in form to those in figure 4. The zero time represents the initiation of the fifth thalamic stimulus in a train of eight. In this experiment both the monosynaptic reflex and the polysynaptic reflex were depressed during the interval occupied by the train of thalamically evoked relayed volleys.

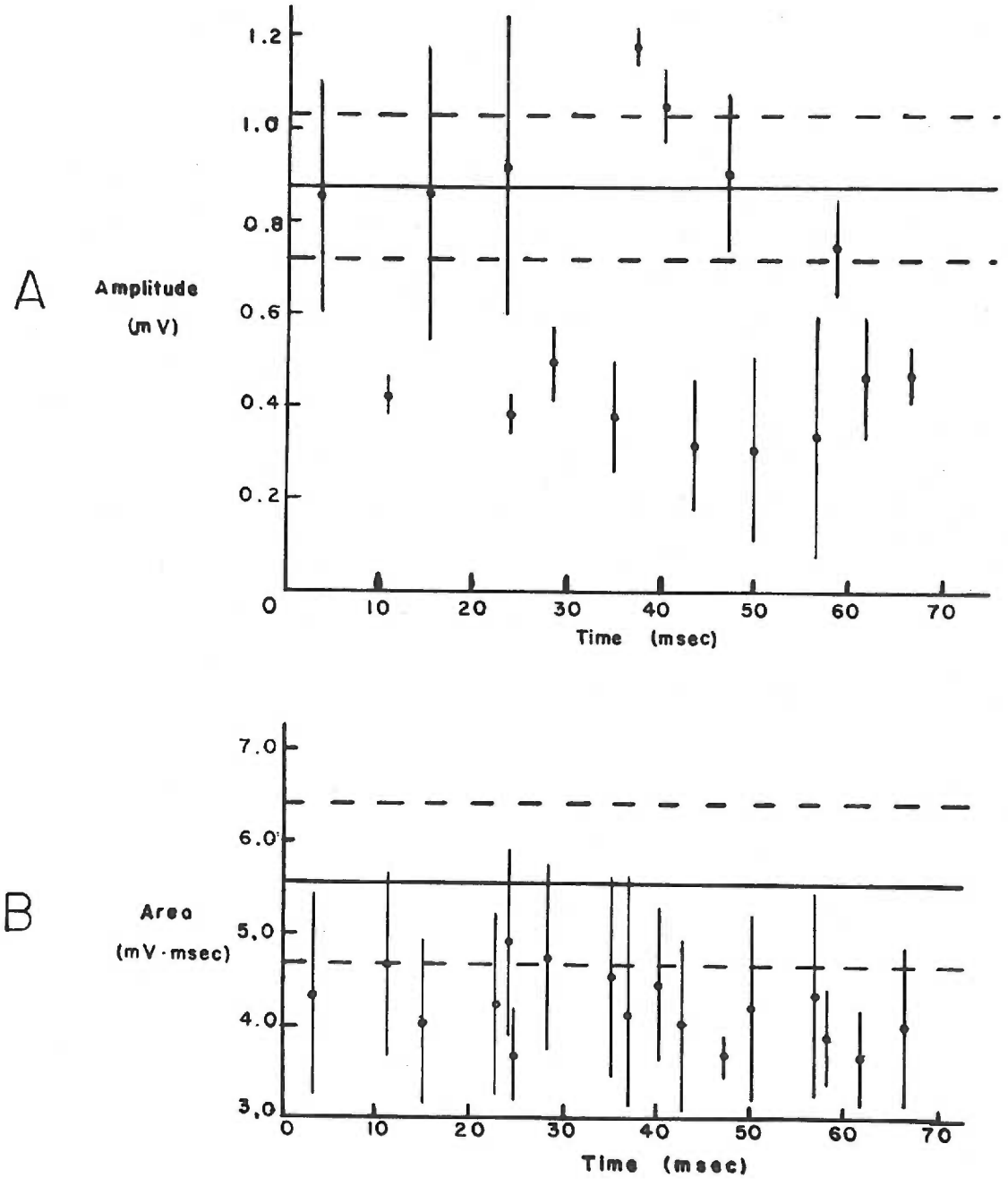
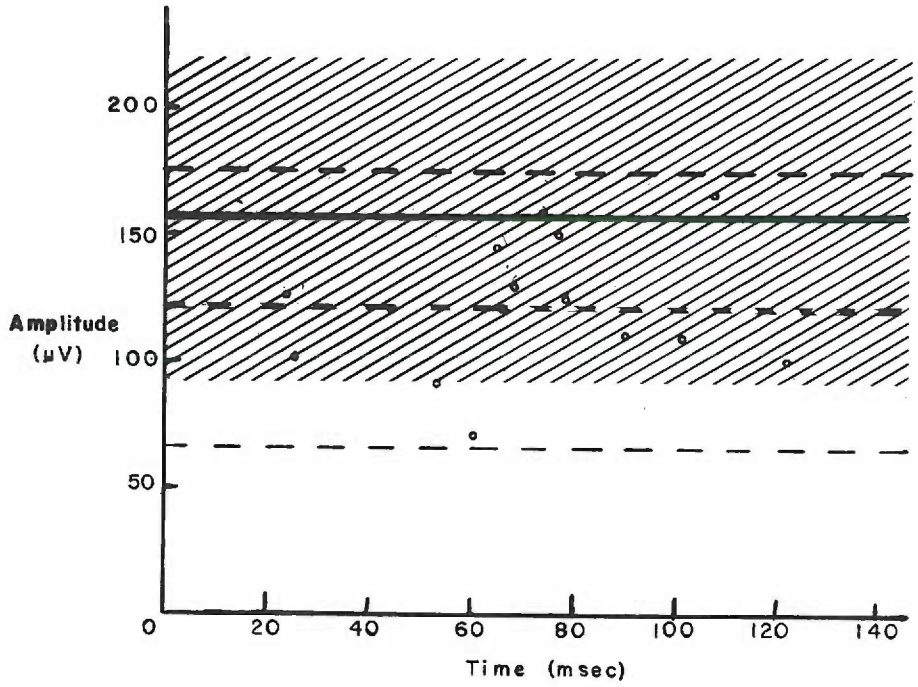

A
Amplitude
(mV)
1.2
1.0
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
70
Time (msec)
B
Area
(mV·msec)
7.0
6.0
5.0
4.0
3.0
0
10
20
30
40
50
60
70
Time (msec)

Figure 6

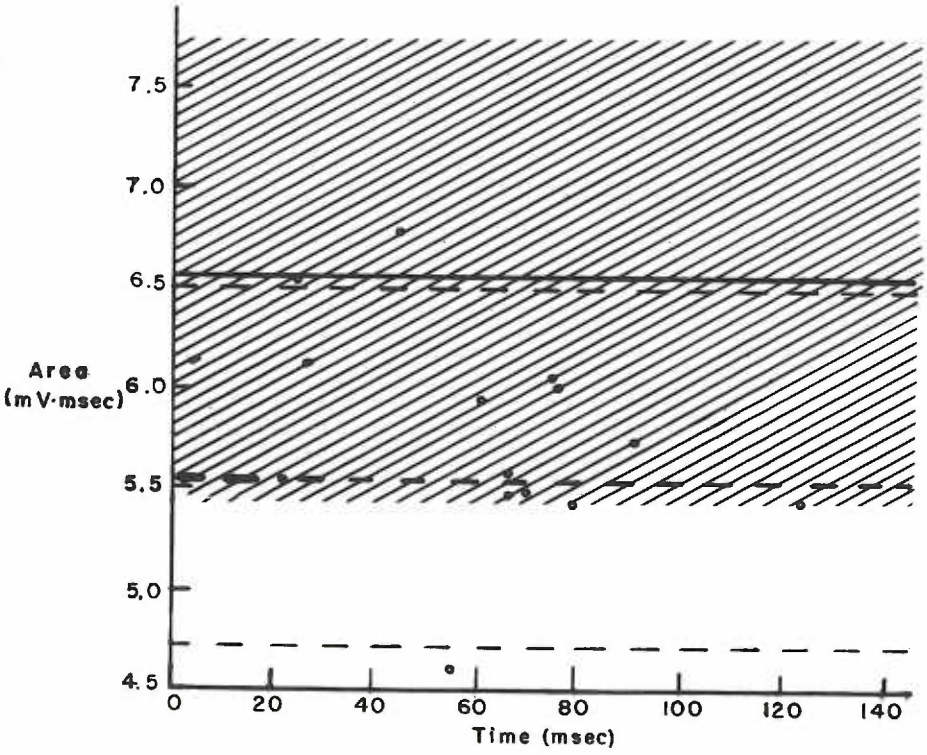
GRAPHIC ILLUSTRATION OF THE EFFECTS OF SPINDLING ON SPINAL REFLEXES IN A SINGLE CAT.

Again Graph "A" illustrates the changes in amplitude of the monosynaptic reflex during the period of a single relayed volley accompanying spontaneous spindle waves. Graph "B" illustrates changes in the areas of polysynaptic reflexes. The first downward deflection of the relayed volley is taken to occur at zero time. The relayed volley persists for about 25-30 msec. The heavy dotted line represents the mean of the conditioned reflex discharges. The two dotted lines on either side of the mean express two standard deviations above and below this mean. The heavy solid line is the mean of the unconditioned responses. The hatched lines express two standard deviations above and below this mean. Each plotted point illustrates the amplitude or area of a single reflex evoked at a specific time interval following the initiation of the second relayed volley accompanying spontaneous spindle waves. In this preparation spindling depressed both the monosynaptic and polysynaptic reflex discharges.

A


200
150
100
50
0
Amplitude
(μV)
20
40
60
80
100
120
140
Time (msec)

B


7.5
7.0
6.5
6.0
5.5
5.0
4.5
Area
(mV·msec)
0
20
40
60
80
100
120
140
Time (msec)

graph "B" of figure 4. Relayed cortico-spinal volleys initiated by thalamic stimulation depressed the polysynaptic portion of the reflex discharge in four cats (Exp. 2, 6, 7, and 9). The results from Experiment 9 are illustrated on graph "B" of figure 5. The polysynaptic reflex was facilitated in one spindling cat (Exp. 11) but depressed in two others (Exp. 12 and 14). Cortico-spinal conditioning, whether initiated by thalamic stimulation or spindling, did not alter the areas of polysynaptic reflexes in two cats (Exp. 10 and 13).

It was a general rule that if conditioning changed the magnitude of one portion of the reflex in one way, the magnitude of the other portion of the reflex was changed the same way during conditioning. In Experiment 11, both the monosynaptic and polysynaptic portions of the reflex discharge were facilitated. In Experiments 9, 12, and 14, both the monosynaptic and polysynaptic reflexes were depressed. There were exceptions to this rule, however. In Experiments 6, 10, and 13, only one portion of the reflex was changed during conditioning. In Experiment 7, the monosynaptic reflex was facilitated while the polysynaptic reflex was depressed as a result of the cortico-spinal volleys evoked by thalamic stimulation. In Experiment 8, the monosynaptic reflex was depressed, but the polysynaptic reflex was facilitated under apparently similar circumstances. These observations indicate that excitabilities of motor neurons and interneurons in the two reflex pathways can be altered independently by conditioning volleys of cortico-spinal activity.

The comparison of these aggregate values for all reflex responses by this type of analysis indicates that relayed volleys in cortico-spinal fibers altered the level of motor neuron excitability. While

such a comparison of aggregate values can reveal trends in excitability over the entire testing period, it cannot reveal the presence or absence of cyclic alterations in excitability temporally related to the relayed cortico-spinal volleys. Such information could be gained only from the study of reflex responses evoked at numerous short intervals before, during, and after a single relayed volley. The information bearing on this point is illustrated in the graphs presented in figures 4 and 5. In each of these experiments, the relayed volley was initiated approximately ten msec. after the thalamic shock at time zero, and endured for 20-25 msec. No significant alterations in reflex amplitude or area occurred which differentiated this period from that period immediately preceding or following the relayed volley.

It was immaterial which of the waves of a train was selected for temporal fractionation; no significant alterations in reflex response could be detected in relation to any single relayed volley. Similar results have been obtained by fractionation of the relayed volleys following both augmentation and spindling. It would thus appear that the influence of the relayed volleys persists uniformly throughout the period of the conditioning train.

While inspection of the results fails to reveal cyclic alterations in excitability temporally related to the relayed cortico-spinal volley, it is possible that a temporal relation does exist. The monosynaptic reflex illustrated graphically in figures 4 and 5 appears to be facilitated about 30 msec. after the initiation of a thalamic stimulus. It is important to test whether this apparent facilitation is real or simply a variation of excitability unrelated temporally to the relayed cortico-

spinal volley.

This possibility has been tested by subjecting to an analysis of variance, the standard deviations of amplitudes and areas of reflex discharge recorded at specific times during and after an evoked relayed volley. It has already been shown that conditioning activity will alter the mean level of motor neuron excitability. It is reasonable to assume that conditioning could result in changes of the scatter of excitabilities about the mean conditioned level of excitability. Changes in scatter of excitabilities temporally related to a relayed volley of cortico-spinal activity would be detected by a significant "F" number obtained by an analysis of the variance.

This analysis was performed on the results obtained from responses initiated in relation to a single volley of cortico-spinal activity evoked by stimulation of the thalamus. The "F" numbers obtained from Experiments 4-10 for these analyses are listed in column "G" of Table I. None of these values is significant. Again, it made no difference which wave of relayed cortico-spinal activity was chosen for fractionation.

Since it may be considered that the technique used for temporal grouping of the standard deviations is rather artificial, analyses were performed on standard deviations grouped in 20 msec. intervals, 30 msec. intervals, and grouped randomly with no regard to the temporal relationships of the responses to a relayed cortico-spinal volley. No significant changes of the scatter of excitabilities could be detected. This indicates that the scatter of excitabilities of conditioned motor neurons does not vary significantly throughout the interval between relayed volleys.

Individual reflex discharges were subjected to the same type of analysis in Experiment 14. In this experiment, adequate temporal fractionation of the second relayed volley accompanying spindle bursts was achieved. The results of the analysis indicate again that significant variations in either mean level or scatter of excitabilities did not occur.

This type of analysis clearly demonstrates that no significant cyclic variations in motor neuron excitability occurred which were temporally related to a volley of cortico-spinal activity. This type of analysis also confirms the observation that the influence of the relayed volley persists uniformly throughout the period occupied by the conditioning train. Unfortunately no information is available to indicate how soon the conditioning effects were present after the initiation of the first volley of cortico-spinal activity, and no information is available to indicate how long the alteration of motor neuron excitability persisted after the end of the train of conditioning volleys.

DISCUSSION

The results of these experiments can be summarized in a few simple statements. Thalamic stimulation and spontaneous spindling which evoke relayed volleys of cortico-spinal activity may alter the level of excitability of spinal cord motor neurons. This was evidenced by changes in the amplitudes of monosynaptic reflexes and in the areas of polysynaptic reflexes during conditioning. Conditioning could either raise or lower the excitability of motor neurons, but it was impossible to predict from experiment to experiment which form of alteration would occur. The interval between conditioning and testing appeared to make no difference, and the regulatory effects persisted uniformly throughout the interval occupied by the train of conditioning volleys.

Are the results valid, or are they some sort of odd experimental quirk? All results presented are based on careful statistical analysis. All analyses performed are based on the null hypothesis, i.e., one population is no different from any other population of comparable entities. An example in terms of the present experiments would be: the population of conditioned responses is no different from the population of unconditioned responses. If a particular test shows that the populations are different, it must be proved that they are not different. Conversely, if testing shows the populations to be the same, it must be proved that they are not the same. Statistical analysis has failed to support the null hypothesis in a majority of the experiments. It must be concluded then, that the results are valid and give a true indication of the changes

of the excitability of spinal motor neurons during the types of conditioning procedures employed in the present series of experiments.

Inter-animal Variations

The attempt to understand the meaning of the results constitutes a rather complex problem. It would appear that presumably identical procedures could, in one animal, cause one effect and, in another animal, cause the totally opposite effect.

A possible explanation of this phenomenon can be gained by an examination of the experimental procedure. All preparations were unanesthetized and were not rendered immobile by using curare-like drugs. Because peripheral nerve stimulation did cause movements and a large movement artifact, it was necessary to immobilize both hind limbs securely. This was accomplished either by taping the two hind limbs to upright rods fastened to the top of the work table, or by fastening both hind limbs to the legs of the working table by means of heavy cord. Fastening the cat in this manner stretched both lower extremities and undoubtedly activated to a high degree stretch receptors of both limbs. The cord and tape must have activated cutaneous receptors. The effects of this sensory activation on cord interneurons is unknown, and was certainly not the same from experiment to experiment as it was impossible to duplicate this experimental variable from day to day.

The reversal of cortico-spinal regulatory effects from cat to cat could be explained because of this experimentally induced variable. The level of interneuron excitability prior to the arrival of cortico-spinal conditioning could conceivably add to the dominant effect present in the

conditioning volley; however, it is doubtful that this adequately explains the reversal noted. Preston and Whitlock (38) found that stretching of the hind limbs of their preparations did change the absolute level of unconditioned motor neuron excitability in their preparations, but didnot alter the basic pattern of cortico-spinal influence. It appears doubtful that the preconditioning level of excitability of the interneuron and motor neuron could be solely responsible for the reversal in conditioning effects seen in different preparations.

Other possible explanations may be found by examining the possible pathways over which the conditioning activity may have reached the cord. In addition to the direct cortico-spinal fibers, the large mid-brain lesion also spared cortico-bulbar pathways. Impulses in either of these pathways could have excited elements of the brainstem reticular formation over cortico-bulbar fibers themselves or over collaterals from cortico-spinal fibers, both of which terminate in the reticular formation (39, 42). Conditioning activity then could reach the cord over cortico-spinal fibers and reticulo-spinal pathways. It is possible that the degree of reticular influence could vary from cat to cat because of differences in the cats' general condition. Blood loss, anoxia, and shock may impair reticular function and this influence could certainly vary from animal to animal.

Although the lesion was placed in such a fashion as to spare cortico-spinal and cortico-bulbar fibers, the lesion also spared a small portion of the periaqueductal grey matter. The experience which has been gathered with the use of this mesencephalic lesion in this and other laboratories has indicated that the probability of the development

of cerebral edema is greatly reduced if the aqueduct of Sylvius is not destroyed by the lesion. In order to prevent encroachment of the lesion on the aqueduct, a small amount of central grey substance was left intact to forestall the appearance of cerebral edema. The amount of this portion of the brain-stem spared from day to day was by no means constant. It is possible that the different regulatory effect seen in different cats could in part have been due to the day to day variation in the amount of periaqueductal grey matter available for conduction of descending influences.

One other important pathway was spared by the lesion. The medial one-fifth and the lateral one-fifth of the cerebral peduncle contain cortico-pontine fibers which may influence cerebellar function over relays through the pontine nuclei. While the lesion usually encroached upon the lateral portion of the peduncle, the medial portion of the peduncle was spared routinely. Thus, a major source of cerebral input to the cerebellum was left intact. Since no attempt was made to interrupt pathways conveying descending cerebellar influences, it is possible that regulatory influences were mediated in part through the cerebellum. Cerebellar function may, in part, explain the inhibition of motor neurons observed (43). It is also quite possible that the degree of cerebellar influence could have varied from day to day. Two factors could have been responsible for a variable degree of cerebellar influence. The input to the cerebellum from the cerebral cortex could have varied from day to day depending upon the extent of the mid-brain lesion. Cerebellar function could have varied from day to day due to the general condition of the cats. The cerebellum could have been depressed in anoxic cats (45).

Since the animals breathed spontaneously, any number of undetected changes in the animals could have occurred which resulted in a relative anoxia of a magnitude sufficient to have depressed cerebellar function.

Any or all of the possible explanations offered could have caused the different effect seen in different cats. It is impossible to state which mechanism was the most important. It is more reasonable to assume that conditioning effect observed was due to varying degrees of interaction between all the factors mentioned. It is readily apparent that a number of pathways is available for mediation of the conditioning activity.

Mechanism of Conditioning

In the majority of the present experiments, conditioning either raised or lowered the excitability of spinal cord motor neurons. The mechanism by which the excitability of motor neurons was raised appears to be fairly clear. Thalamic stimulation and spontaneous spindling excited cortical elements that subserve a facilitatory influence on motor neurons. This facilitatory influence must have been mediated over pathways spared by the mesencephalic lesion. At this point, it is tempting to conclude that the cortico-spinal pathway was the only one involved since it is known that the cortico-spinal tract conveys facilitatory impulses (27,38). However, depression of motor neuron excitability was also noted as a result of volleys of conditioning activity.

It is more difficult to account for the mechanisms involved in the depression of motor neuron excitability observed in the present series of experiments. The magnitude of spinal reflexes may be depressed if the motor neurons or interneurons are inhibited, or if the incoming

sensory volley is occluded.

It is possible that cortico-spinal volleys could have occluded incoming sensory volleys at interneurons shared by cortico-spinal fibers and primary afferent fibers. If such occlusion had occurred, the evidence of its existence would have appeared in the form of depression of the polysynaptic component of the reflex discharge. Such occlusion could depress the amplitude of the monosynaptic reflex discharges only if motor neurons were activated by the cortico-spinal volley itself. In no experiments were motor neurons activated by volleys of cortico-spinal activity. Consequently, it may be concluded that the process of occlusion is not involved in the depression of reflex activity noted in these experiments.

It appears more likely that thalamic stimulation and spontaneous spindling activated cortical elements which subserve an inhibitory influence on the excitability of spinal cord motor neurons. This inhibitory influence must have been mediated over pathways spared by the midbrain lesion. This hypothesis is supported by Preston and Whitlock (38) who recorded ipsp's from motor neurons following single cortical shocks in pyramidal monkeys.

It is unlikely that the inhibitory influences were mediated over the cortico-spinal tract. Lloyd (27) observed only facilitation of motor neurons following stimulation of isolated bulbar pyramids. Preston and Whitlock (38) were unable to prove that the inhibition observed in their study was mediated by the cortico-spinal tract. Thus it is more likely that inhibitory influences were mediated over pathways other than the cortico-spinal tract.

Two possible pathways appear to be the most likely to be responsible for mediation of inhibition. The caudal portions of the medullary reticular formation are known to have an inhibitory influence on spinal motor neurons (26, 31). Numerous collaterals of cortico-spinal fibers terminate in this region of the reticular formation (39, 42). Thus, one possible pathway for inhibition is over reticular-spinal fibers. Another important pathway of inhibition is through the cerebellum (26, 43). It is quite possible that the cerebellum was activated by some of the conditioning volleys which were relayed over pontine nuclei. The cerebellar inhibition would then have been relayed over reticulo-spinal fibers. Either, or more likely, both pathways could have mediated the inhibitory influence observed.

Since both facilitatory and inhibitory effects were noted, it can be postulated that the effect seen in any one animal represents the net effect of opposing facilitatory and inhibitory influences on the motor neuron pools tested. Apparently this net effect can differ from day to day, and can independently alter the level of excitability of the motor neurons and interneuron which constitute the monosynaptic and polysynaptic reflex pathways.

Another question concerns the persistence of the regulatory effects. In all cats, the regulatory effect persisted uniformly throughout the interval occupied by a train of conditioning activity. One possible explanation is that a cortico-spinal volley was temporally dispersed over a duration sufficient to cause the prolonged regulation of motor neuron excitability observed. Lloyd (27) observed that one pyramidal volley which was temporally dispersed over a 7-8 msec. period at the level of

the lumbar cord resulted in facilitation of motor neurons which had just previously been conditioned by 2-5 pyramidal volleys. This facilitation persisted for 30-40 msec. Thus, it is possible that a volley of cortico-spinal activity evoked by thalamic stimulation or spontaneous spindles which is dispersed over an interval of 35-40 msec. at the lumbar cord could result in the regulatory effects which persist for at least 100-130 msec. following a thalamic stimulus or spindle wave.

Another possible explanation is again based on the assumption that the conditioning volleys activated pathways other than the cortico-spinal tract. It would appear that conditioning activity arriving at the cord is dispersed temporally to a great extent by a multiplicity of synaptic delays, and because the activity arrives at the cord over pathways which conduct at vastly differing velocities. If this is true, the conditioning volley reaches the cord as a highly asynchronous temporally dispersed train of activity arriving over a variety of different pathways. This temporally dispersed activity could maintain the level of motor neuron activity over a long interval of time.

It is possible that conditioning activity was temporally dispersed over pathways involving the cerebellum, and in particular, the reticular formation. This hypothesis is supported by the observation that repetitive stimulation of the reticular formation has a prolonged influence on the excitability of spinal motor neurons. This influence will persist for as long as 1-2 seconds following reticular stimulation (2, 21). If it is assumed that thalamic stimulation and spontaneous spindles activated reticulo-spinal pathways, the prolonged discharge from these elements could have maintained the level of motor neuron excitability.

Preston and Whitlock (38) have suggested this mechanism to explain the secondary period of facilitation observed in their study. They found that single shocks to the medullary reticular formation resulted in facilitation of the monosynaptic reflex after a latency almost identical to the secondary facilitation after a single cortical stimulus. The even more persistent regulatory influence observed in the present experiments could have been due to rhythmic 8-12 per second activation of reticular units.

It is evident that the level of excitability of spinal motor neurons is altered by repetitious cortical activity that evokes volleys of cortico-spinal activity. It is not likely that these regulatory influences were mediated solely over direct cortico-spinal pathways. Moreover, it is impossible to delineate which effect is primarily cortico-spinal. An analysis of pathways available reveals that the conditioning activity could have been mediated over the cortico-spinal tract, reticulo-spinal pathways, and over pathways involving the cerebellum. Facilitation of motor neurons was observed in those cats in which the predominant effect impinging on the motor neurons and interneurons of the cord over the multiple pathways mentioned above was facilitatory. Inhibition was observed in those preparations in which the predominant effect was inhibitory. In an intact functioning animal, these effects are undoubtedly blended in such a way that smooth, purposeful movements result.

SUMMARY

1. The effects of activation of the cortico-spinal system on the excitability of spinal cord motor neurons have been studied in unanesthetized cats with large lesions of the mesencephalic tegmentum designed to spare cortico-spinal and cortico-bulbar pathways of the cerebral peduncle.

2. Conditioning volleys of cortico-spinal activity were evoked by stimulation of the ventro-lateral thalamus and by the spontaneous spindle mechanisms, both operating at the cortical level. Changes in the excitability of spinal motor neurons as a result of conditioning were detected by comparing the magnitudes of spinal reflexes obtained during conditioning with the magnitudes of unconditioned reflexes.

3. Thalamic stimulation and spontaneous spindle bursts which evoked volleys of relayed cortico-spinal activity raised the level of motor neuron excitability in some cats, but lowered the level of motor neuron excitability in other cats.

4. Observations indicate that the conditioning volleys could independently alter the excitability of motor neurons and interneurons that make up the monosynaptic and polysynaptic reflex pathways.

5. No cyclic variations of motor neuron excitability related temporally to a relayed volley could be detected. It appeared that the conditioned level of motor neuron excitability persisted uniformly throughout the interval occupied by a train of conditioning volleys.

6. The results have been extensively discussed. It is likely that the conditioning effect seen represents the net effect of opposing facili-

tation and inhibition converging on the interneuron and motor neuron pools of the cord from a number of different sources activated by thalamic stimulation and spontaneous spindles.

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APPENDIX

The following are the analysis of variance tables for the single classification analyses performed on the results from Experiments 1-3 and 11-14. "t" scores for each experiment are tabulated at the bottom of each analysis of variance table.

July 3, 1959

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	44.52	1	44.52	454.85
WITHIN	5.17	56	0.098	$F_{95}(1, 53) = 4.04$
TOTAL	49.69	57		

$t = \sqrt{454.85} = 20.13$
$t_{95}(56) = 2.01$

July 9, 1959

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	9.3	1	9.3	21.13
WITHIN	43.7	99	.44	$F_{95}(1, 99) = 3.94$
TOTAL	53	100		

$t = \sqrt{21.13} = 4.59$
$t_{95}(99) = 2.00$

July 13, 1959

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	1.01	1	1.01	12.62
WITHIN	3.74	46	.08	$F_{95}(1, 47) = 4.06$
TOTAL	4.75	47		

$t = \sqrt{12.62} = 3.55$
$t_{95}(46) = 2.02$

August 31, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	23,144	1	23,144	8.17
WITHIN	56,634	20	2,831.7	$F_{95}(1, 20) = 4.35$
TOTAL	79,778	21		

$t = \sqrt{8.17} = 2.86$
$t_{95}(20) = 2.09$

August 31, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	6.69	1	6.69	9.16
WITHIN	14.68	20	0.73	$F_{95}(1, 20) = 4.35$
TOTAL	21.37	21		

$t = \sqrt{9.16} = 3.04$
$t_{95}(20) = 2.09$

October 2, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	8630	1	8630	8.65
WITHIN	26946	27	998	
TOTAL	35476	28		

$t = \sqrt{8.65} = 2.94$
$t_{95(27)} = 2.052$

October 2, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	8.96	1	8.96	19.91
WITHIN	12.06	27	0.45	$F_{95(1, 27)} = 4.20$
TOTAL	21.02	28		

$t = \sqrt{19.91} = 4.46$
$t_{95(27)} = 2.052$

October 6, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	32,321	1	32,321	20.12
WITHIN	51,431	32	1,608	$F_{95}(1, 32) = 4.15$
TOTAL	83,752	33		

$t = \sqrt{20.12} = 4.37$
$t_{95}(32) = 2.04$

October 6, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	0.62	1	0.62	4.43
WITHIN	4.57	32	0.14	$F_{95}(1, 32) = 4.15$
TOTAL	5.19	33		

$t = \sqrt{4.15} = 2.037$
$t_{95}(32) = 2.042$

October 15, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	38,920,990	2	19,460,495	21,385
WITHIN	48,204	52	910	$F_{95}(2, 52) = 4.02$
TOTAL	38,969,194	54		

Comparing mean value of conditioned responses with mean value of unconditioned response.

$t = 15.40$, $t_{95}(52) = 2.02$

Comparing mean value of conditioned response with mean value of responses obtained after pyramids were coagulated.

$t = 31.30$, $t_{95}(52) = 2.02$

October 15, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	1.53	2	0.76	1.76
WITHIN	22.16	52	0.43	$F_{95}(2, 52) = 4.02$
TOTAL	23.69	54		

Comparing conditioned mean with mean of unconditioned responses.

$t = 4.53$, $t_{95}(52) = 2.02$

The following are the analysis of variance tables for the analysis of the scatter of excitabilities performed on the data from Experiments 4-10 and Experiment 14. The "r" value obtained from each sign test (see text for description) is tabulated at the bottom of each analysis of variance table.

July 15, 1959

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	.01	5	.002	0.2
WITHIN	.11	12	.01	$F_{95}(5, 12) = 4.68$
TOTAL	.12	17		

Sign test
N = 18 r(-) = 0 Significant increase at 95% level.

July 23, 1959

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	.040	5	.008	2.96
WITHIN	.030	11	.0027	$F_{95}(5, 12) = 3.20$
TOTAL	.070	16		

Sign test
N = 17 r(-) = 3 Significant increase at 95% level.

July 29, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	23,743.1	5	4760	2.39,
WITHIN	23,896.78	12	1991.39	$F_{95}(5, 12) = 3.11$
TOTAL	47,641.88	17		

Sign test
N = 18 r(+) = 7 Significant increase at 95% level.

July 29, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	0.07	5	0.014	1.00,
WITHIN	0.17	12	0.014	$F_{95}(5, 12) = 3.11$
TOTAL	0.24	17		

Sign test
N = 18 r(+) = 0 Significant decrease at 95% level.

September 15, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	2188.66	5	437.73	2.03, $F_{95}(5, 12) = 3.11$
WITHIN	2586.89	12	215.57	
TOTAL	4775.55	17		

Sign test
N = 18 r(-) = 2 Significant increase at 95% level.

September 15, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	0.13	5	0.026	0.34, $F_{95}(5, 12) = 3.11$
WITHIN	0.21	12	0.075	
TOTAL	0.34	17		

Sign test
N = 18 r(+) = 0 Significant decrease at 95% level.

September 22, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	750.1	5	150.05	2.22, $F_{95}(5, 11) = 3.20$
WITHIN	746.72	11	67.88	
TOTAL	1496.82	16		

Sign test
N = 17 r(+) = 4 Significant decrease at 95% level.

September 22, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	0.09	5	0.018	1.28, $F_{95}(5, 11) = 3.20$
WITHIN	0.15	11	0.014	
TOTAL	0.24	16		

Sign test
N = 17 r(-) = 0 Significant increase at 95% level.

September 25, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	8270.9	5	1654.2	0.68,
WITHIN	24194.2	10	2419.42	$F_{95}(5, 10) = 3.33$
TOTAL	32465.1	15		

Sign test
N = 17 r(+) = 3 Significant decrease at 95% level.

September 25, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	0.36	5	0.07	2.26,
WITHIN	0.31	10	0.031	$F_{95}(5, 10) = 3.33$
TOTAL	0.67	15		

Sign test
N = 17 r(+) = 0 Significant decrease at 95% level.

September 29, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	10,949.98	5	2189.90	1.75,
WITHIN	11,264.68	9	1251.60	$F_{95(5, 9)} = 3.48$
TOTAL	22,211.66	14		

Sign test
N = 15 r(+) = 3 Significant decrease at 95% level.

September 29, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	0.34	5	0.068	0.567,
WITHIN	1.08	9	0.12	$F_{95(5, 9)} = 3.48$
TOTAL	1.42	14		

Sign test
N = 15 r(+) = 7 Significant increase at 95% level.

October 15, 1959
Monosynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	8590	4	2147.5	0.234, $F_{95}(4, 10) = 3.48$
WITHIN	91674	10	9167.4	
TOTAL	100264	14		

October 15, 1959
Polysynaptic reflex

	SUMS OF SQUARES	df	MEAN SQUARES	F
CATEGORY MEANS	1.42	4	0.33	0.39, $F_{95}(4, 10) = 3.48$
WITHIN	8.52	10	0.852	
TOTAL	9.94	14		