RUBROSPINAL INFLUENCES ON SELECTED ALPHA MOTONEURON POPULATIONS OF THE DOG

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CHAPTER I

INTRODUCTION

As early as 1906, Sherrington described spinal cord reflexes which are now known to be capable, at a segmental level, of providing a functional basis for maintenance of posture, avoidance of noxious stimuli, and certain stereotyped pacing movements. With the advent of anatomical degeneration studies and electrophysiological techniques developed in the last few decades, attention has been directed toward understanding how these basic mechanisms might work in an integrative manner to provide purposeful control of somatic musculature. Important in this regard is the study of supraspinal control over the motoneurons and basic neuronal circuits of the spinal cord.

The rubrospinal tract provides one of the major descending pathways by which control from higher centers may be exerted over the more basic neuronal circuitry of the spinal cord. The course of the rubrospinal tract has been traced to the lowest segment of the lumbar enlargement in several animals, although the terminal ramifications of the fibers have only recently been studied with the aid of silver impregnation methods in the macaque (sub-human primate) and the cat. The rubrospinal tract arises from the red nucleus (RN) in the midbrain, immediately crosses to the contralateral side in the mesencephalic decussation, and eventually passes into the lateral funiculus of the spinal cord.

Of great functional significance are the afferent somatotopic

connections of the RN from the cerebral cortex and the cerebellum. These connections indicate that the rubrospinal tract may be of importance as an efferent pathway for cerebellospinal impulses as well as an "extrapyramidal" pathway from the motor cortex. Thus the RN appears to be an important segment of the sensorimotor system in which signals, not only from the cerebral cortex, but also from the cerebellum and other structures converge and are integrated at the level of the brain stem.

Most electrical stimulation experiments on the RN have been conducted on the cat. Pompeiano (1957) found that stimulation of the RN induced a flexion of either the anterior or the posterior contralateral limb, depending upon the part stimulated. Thulin (1963), by using the monosynaptic reflex testing in decerebrate and decerebellate cats, obtained variable results with mostly facilitation of the flexor motoneurons, but little effect on the extensors. Other workers have utilized intracellular electrodes on intact animals under light anesthesia and obtained results showing that excitatory postsynaptic potentials (EPSP's) predominate in flexor motoneurons whereas inhibitory postsynaptic potentials (IPSP's) may occur more frequently in extensor motoneurons.

To date, no studies of electrophysiological investigations of the rubrospinal system influence on spinal motoneurons have been reported in the dog. The research in this dissertation is designed to study the influences of rubrospinal tract volleys on spinal motoneuron populations in this species.

CHAPTER II

REVIEW OF LITERATURE

This review of literature is concerned only with that portion of the available information which best represents the current status of the RN as a sensorimotor center of the brain. Many of the earlier and sometimes controversial publications have been examined only superficially in light of the more conclusive experiments of the last eighteen years. For a more extensive synopsis of the literature prior to 1967 concerning overall structure and function of the RN, the reader is referred to an excellent review by Massion (53).

The following résumé of literature is organized into sections concerning: 1) structure and organization of the RN; 2) anatomical and functional considerations of the RN with the cerebellum, cerebral cortex, and diencephalon; 3) anatomical and functional considerations of the rubrospinal tract; and 4) use of the monosynaptic reflex as a means of testing alpha motoneuron excitability in the spinal cord.

Structure and Organization of the Red Nucleus

The RN is rather sharply defined as a separate anatomical entity within the rostral portion of the mesencephalic tegmentum, due to its color (said to be due to particular vascularity), and its "capsule" which is formed by fibers of the brachium conjunctivum which surround and traverse it (29). Three types of cells may be found in the RN of

all mammals: large (50-90μ), medium (30-40μ), and small (20μ), (6,14, 56,59,61,64,66,67). In all mammals studied thus far, the large cells are more compact in the caudal part of the RN, thereby giving basis for distinguishing between a caudal "magnocellular" part and a more rostral "parvocellular" part (6,14,56,59,61,64,66,67). In the evolution of mammals there appears to be a regression of the magnocellular portion in primates and anthropoids.

In the cat, anatomical studies by Davenport and Ranson (14), and more recently Brodal and Gogstad (6), indicate that the transition from a magnocellular to a parvocellular part of the nucleus is diffuse. Even in the caudal extremity of the magnocellular part there are some small cells, and large cells occur in considerable numbers at rostral levels. Along with these changes in cellular composition there is a progressive change from a compact to a more loose reticular structure of the nucleus. Recent physiological evidence indicates however that it is "fictitious" to extend these morphological divisions into having functional significance (67). It has been shown that cells of all sizes receive fibers from the somatic motor cortex (67) and project to the spinal cord (61) and cerebellum (56) regardless of whether these are localized at rostral or caudal levels.

In the monkey, the larger cells are concentrated in the caudal part of the RN and constitute a well delineated "magnocellular portion" which gives rise to purely crossed rubrospinal fibers. The smaller neurons are concentrated more rostrally in the "parvocellular portion" of the RN and discharge exlusively to the ipsilateral caudal olive (53,64). This is in contrast to the more diffuse origin of the efferents of the cat RN and suggest to Poirier (64) that from one species (cat) to the other

(monkey) a migration and specialization of the nervous elements take place within the RN in relation to the organization of the mechanisms controlling peripheral motor activity.

In man, the nucleus consists of three parts, of which the medial and lateral are greatly enlarged while the large cells of the posterior part are markedly reduced in number (59). Thus the human RN may be said to consist essentially of a parvocellular part (53). As with the monkey, each of the parts of the RN appear to be related to a descending fiber tract (59).

A small lateral extension of the nucleus is seen in ungulates and carnivores (53) forming a group of rather densely packed small cells corresponding to the nucleus minimus of Von Monakow (88). Pompeiano and Brodal (61) demonstrated in the cat that these neurons project only to the cervical segments of the spinal cord.

Red Nucleus and Cerebellum Considerations

The fact that the principal afferent fibers to the red nuclei arise from the deep cerebellar nuclei and pass via the brachium conjunctivum has long been known (9), but precise information concerning the topographical arrangement of fibers within the brachium conjunctivum to cerebellar nuclei has only recently been elucidated. In his review of literature prior to and including his 1966 publications, Massion (53) concluded that

> there is no doubt that the nucleus interpositus and the dentate nucleus both send fibers to the red nucleus in all animals except man, where, according to Hassler the projection is derived from the dentate nucleus only.

Most workers agree that, in the cat, cerebellar input to

contralateral RN neurons is accomplished primarily through the interpositus (IP) nucleus (1,12,13). Angaut (1) found that only those fibers from the IP nucleus terminate in the magnocellular RN. He did however, observe a small amount of preterminal degeneration in the prerubral region of the zona incerta following lesions of the dentate nucleus. Courville et al. (13) and Conde et al. (11) have concluded from their studies in the cat that some of the small neurons in the more anterior parvocellular portion of the RN have an afferent and efferent organization different from the other RN cells. These small neurons appear to receive excitatory projections from the dentate nucleus of the cerebellum and send axons rostrally into the nucleus ventralis of the thalamus.

Thus, it would appear in the cat as if cerebellar input to RN neurons directly involved in the rubrospinal system are by way of the IP nucleus. A relatively small input from the dentate nucleus is probably present only to those small neurons of parvocellular RN which project rostrally to the nucleus ventralis of the thalamus (11,13).

A somatotopic organization of the cerebellar projection to the RN by way of the IP nucleus has been demonstrated (12,48). Projections from the anterior lobe of the cerebellar cortex are such that the anterior lobules of the intermediary zone relay to the contralateral RN posterior limb area by way of the anterior IP nucleus. Those from the posterior lobules of the anterior lobe project to the contralateral RN anterior limb area by way of a relay in the caudal part of the anterior IP nucleus. A reversed arrangement is found concerning the projections from the paramedian zone of the cortex, which relay in the posterior IP nucleus before reaching the RN (48).

Tsukahara et al. (82) stimulated the IP region with needle

electrodes and was able to induce EPSP's in the RN with a latency of 1.0 msec., indicating a monosynaptic connection. Then starting at 6.7 msec. a large hyperpolarization consistently occurred lasting several ten milliseconds. Since the hyperpolarization had the same null potential as an EPSP, they assumed it to be due to removal of tonically induced EPSP's. Stimulation of the intermediate part of the anterior lobe of the cerebellum produced a similar hyperpolarization with shorter latency (2.2 msec.). These workers concluded that the longer latency of the hyperpolarization from IP stimulation could come from transynaptic activation of Purkinje cells through cerebellar afferent fibers. Thus they suggested that the essential functional feature of the cerebellorubral system appears to be that the IP mediates tonic facilitory influences upon the RN under inhibitory control by the cerebellar cortex (83). This disfacilitation of RN neurons has been confirmed by Toyama et al. (81). They found that the interpositus nuclear cells discharge normally at frequencies of 50-100/sec. and these tonic discharges can be suppressed after cerebellar stimulation (presumedly by Purkinje cell axons).

Efferents from the RN to the cerebellum are: 1) direct to the IP (12,53); 2) indirect by way of the olivary nucleus; and 3) indirect by way of the lateral reticular nucleus (29,53).

Although Brodal and Gogstad (6) demonstrated retrograde degeneration changes in the RN after lesions in the "dentate" nucleus, Courville (12, 13) has shown convincing evidence that the sole projection of the RN to the cerebellum is through the IP nucleus. He explained Brodal and Gogstad's (6) results on the basis that their lesion encroached on the lateral aspect of the IP nucleus anterior which destroys the very region where the rubro-cerebellar tract enters the interpositus. Other workers

substantiate Courville's (12,13) conclusions that in the cat, the RN receives fibers only from the IP nucleus (1). Courville (12) showed that in agreement with physiological work by Maffei and Pompeiano (48) indicating a somatotopical organization within the interposito-rubral projection, there is likewise a similar organization existing in the rubral projection onto the IP. He concluded that the ventrolateral part of the RN (hind limb area) projected onto the rostral most cells of the IP while the dorsomedial part of the RN (forelimb area) projects primarily into areas of the IP situated caudally to those receiving fibers from the hind limb area. Through quantitative estimates, Brodal and Gogstad (6) predict that it is probable there are, in the caudal part of the RN, a certain number of cells which by means of dichotomizing axons projects onto the cerebellum as well as to the spinal cord.

Efferent fibers from the RN passing to specific cerebellar relay nuclei afford other pathways for impulses to enter the cerebellum. Uncrossed rubrobulbar fibers pass from the rostral RN to the dorsal lamella of the principal inferior olivary nucleus of the cat (29). Poirier (64) demonstrated the existence of rubro-olivary fibers in the monkey which originate exclusively in the parvocellular portion of the RN and terminate in the dorsal lamella of the main olive. In man, the central tegmental tract is very large and is apparently in part made up of fibers from the dorsomedial part of the RN which terminate in the principal olive (53,59).

Hinman and Carpenter (29) also demonstrated that a projection passes from the RN to the contralateral lateral reticular nucleus in the cat. Taking into account the special connections of the lateral reticular nucleus and the olivary nucleus with the cerebellar cortex, these

structures provide widespread pathways from the RN to the cerebellar cortex (21).

Red Nucleus and Sensory Motor Cortex Considerations

Using Woolsey's map of the cat's "sensory-motor" (SM) cortex, Rinvik and Walberg (66) demonstrated the existence of a somatotopically arranged cortico-rubral projection in the cat. By utilizing silver impregnation methods to trace degenerating fibers from selected lesions of the cortex they found that corticofugal fibers terminate on cells of all sizes in the rostral as well as in the caudal parts of the ipsilateral RN. The "motor forelimb area" terminates in the dorsomedial part of the RN at caudal levels, in the dorsal region at middle levels, and more diffusely in rostral regions. The "motor hindlimb area" projects primarily to the ventrolateral part of the caudal one-fourth of the RN to the ventral portion of the rostral part. The primary sensory-motor cortex projections were to the ipsilateral RN only and most of the degnerating terminal fibers were in the neuropil of the RN.

In a later study, Rinvik (67) found that the gyrus proreus and the medial aspect of the anterior cruciate gyrus (supplementary motor area) contribute to the cat's cortico-rubral projection system. These projections gave rise to degenerating terminal fibers in both red nuclei, although the number was less in the contralateral nucleus than in the ipsilateral RN. In contrast to those from the primary cortex these degenerating terminal fibers were seen close to the cell somata and processes. The differences between the cortico-rubral projections of the primary and secondary motor areas were indicative to Rinvik (67) of an "elaborate and differential cortical influence on the red nucleus.".

Brindley and Lewis (5) were able to demonstrate somatotopically organized movement patterns in baboons with stimulation of the motor cortex after sectioning the pyramids. It is likely that the RN played an important role in the cortical induction of extrapyramidal pathways involved. The observed organized movements would seem to require a type of somatotopic projection from the SM cortex such as that described by Rinvik (67).

Following the demonstration of a somatotopically organized cortico-rubral projection by Rinvik and Walberg (66), Tsukahara and Kosaka (84) studied the cerebral activation of RN neurons using intracellular recording techniques. They demonstrated monosynaptic EPSP's with a slow time course (4 msec. rise and 25 msec. delay) which they explained as due to cable properties of the dendrites. The cortico-rubral fibers were calculated to conduct impulses at a velocity of less than 20 M/sec. (82,84, 85). More recent work by Tsukahara and Fuller (86) demonstrated that these monosynaptically activated EPSP's were frequently followed by an IPSP. The IPSP was shown to be produced by fast conducting pyramidal fibers, but have a delay that suggests a di- or polysynaptic pathway in the RN. Furthermore, they showed that the pyramidally induced IPSP's were associated with an increase in soma conductance was well as a decrease in simultaneously induced EPSP's from IP nucleus stimulation. In contrast to the IPSP's, the pyramidally induced EPSP's were not associated with significant conductance changes. This data indicates that pyramidally induced IPSP's involve synapses on the soma of the RN neurons whereas the pyramidally induced EPSP's involve synapses located distally on the dendrites of these neurons.

Red Nucleus Connections With the Thalamus and Basal Ganglia

Massion (53) concludes that a small pallido-rubral pathway probably exists in carnivores and the monkey which terminates in the magnocellular portion of the RN although in man the findings are too contradictory to allow any conclusions to be drawn. Papez and Stotler (59) gave convincing evidence for pallidofugal fibers in man which descend into the prerubral field and believe that their observations are in agreement with other workers previous to them. Hinman and Carpenter (29) found a small number of fibers passing from the RN to the globus pallidus and subthalamic nucleus in the cat and suggest that these structures are less important sites of motor integration in carnivores than in man. Ipsilateral efferent rubro-pallidal and rubro-subthalamic fibers have been described in the cat, macaque, and in man (53).

In the cat, RN lesions provoked ipsilateral preterminal degeneration in the parafascicular, centromedian and reticular nuclei of the thalamus (29). Hinman and Carpenter (29) quote observations by Carpenter and Stevens (1957) whereby similar degeneration was not detected following large cerebellar nuclei lesions and conclude therefore that fibers passing to the intralaminar nuclei of the thalamus may originate exclusively in the RN. Poirier and Vouvier (64) were unable to demonstrate any efferent pathways from the RN in the monkey which ascended to the diencephalon. Massion (53) concludes that there are neurons in the parvocellular part of the RN that project to the ipsilateral ventrolateral nucleus. Pompeiano and Brodal (61) observed retrograde changes in the rostral third of the RN after lesions of ascending fibers to higher levels of the brain stem (probably to the thalamus).

Appleberg (2) recorded focal potentials evoked in the RN and the ventrolateral (VL) nucleus of the thalamus by electrical stimulation of the IP nucleus of the cerebellum. They recorded activity in the RN at .49 msec. (18M/sec.) latencies. They interpreted this data to indicate that the path from the cerebellum to the thalamus is direct and not interrupted by a synapse in the RN. Marco (52) recorded unitary postsynaptic potentials in the VL thalamus from RN stimulation which had latencies varying from 18 to 500 msec.. Spike potentials were usually associated with slow postsynaptic potentials. The duration of inhibitory postsynaptic potentials ranged from 50 to 500 msec. depending on the stimulus strength or number of pulses of the stimulating train.

Structure and Function of the Rubrospinal Tract

Anatomy of the Rubrospinal Tract

The rubrospinal tract was first discovered by Von Monakow in the dog in 1883 (88) and has since been described in many species including the rat, rabbit, dog, goat, cat, and monkey (53). Hinman and Carpenter (29) have a relatively recent description of this tract in the cat from their studies with the Nauta-Gygax stain. The tract leaves the caudal pole of the RN as a unified bundle and immediately crosses to the contralateral side in the ventral tegmental decussation. Although several earlier observers had indicated that some uncrossed rubrospinal fibers exist, the more recent observations of Pompeiano and Brodal (61) agree with Hinman and Carpenter (29) that all fibers are crossed.

At the level of the pons these crossed descending fibers move laterally across the dorsal border of the medial lemniscus. At lower

levels the fibers pass dorsolaterally to the superior olivary complex and shift ventrally to traverse the dorsolateral part of the facial nucleus. At medullary levels crossed descending rubral efferent fibers enter the area ventral to the descending nucleus and tract of the trigeminal nerve and can be seen among the cells of the lateral reticular nucleus. These fibers remain ventral to the spinal nucleus and tract of the trigeminal nerve at the level of the pyramidal decussation (56). From a position in the ventral part of the dorsal half of the lateral funiculus in the cervical enlargement, it is gradually displaced in a dorsolateral part of the lateral funiculus close to the most dorsal part of the dorsal horn. A similar distribution of the rubrospinal tract has been described in the rhesus monkey by Kuypers (38).

In man, the rubrospinal tract is usually thought to be rudimentary based on the postulate that only the magnocellular part of the RN projects to the spinal cord. Relatively few large cells are distinguishable in the RN of man. Since it has been shown by Pompeiano and Brodal (61) that, in the cat, small cells in the RN send their axons to the cord, presumedly many of the small cells in the RN of man also send thin and possibly unmyelinated axons to the cord. Such fibers may have escaped recognition in the few studies of the tract conducted in man. Physiological observations in men who have had their pyramid destroyed on one side indicate that mild compensation occurs through some means (8). There is a high probability that this compensation could, in part, be due to the activity of the rubrospinal tract.

Following lesions of the RN in the cat, degenerating fibers and collaterals are seen to enter the gray matter at all levels, although the cervical cord receives the greatest number, and the thoracic cord

receives a lesser number than does the lumbar enlargement (56). The degenerating rubrospinal fibers enter the gray matter mainly laterally in Rexeds laminae V-VII and radiate in a fan-shaped fashion to the lateral half of lamina V, lamina VI, and the dorsal and central parts of lamina VII. The rubrospinal fibers terminate on the somata and along the dendrites of large as well as small nerve cells within the laminae in question. No direct terminations were observed on the ventral motor horn cells (56). It is known however, that the dendrites of lamina IX may project into laminae V, VI, and VII. Therefore the possibility of direct terminals on dendrites of motoneurons cannot be entirely ruled out on anatomical grounds. Kuypers (38) describes a similar termination of rubrospinal fibers in the monkey although his terminology is not as specific (refers to lateral and dorsal parts of the intermediate zone).

Function of the Rubrospinal Tract

The first experiments suggesting a function of the rubrospinal tract involved transection of the brainstem in front of the RN and comparing the reactions of the animal with a transection just caudal to the RN. In a literature review of such studies prior to 1932, Ingram and Ranson (34) found that the extreme decerebrate rigidity which results from a brainstem transection just caudal to the RN is not present in a "hypothalamic cat" (transection just rostral to the superior colliculi and mammillary bodies). Furthermore the "moderate extensor hypertonicity which is present in the hypothalamic cat is sufficiently under control that the animal can crouch in a normal manner", indicating the presence of a motor control center in the region of the RN.

More recent transection experiments suggest that the rubrospinal

system and the pyramidal systems may collaborate in the execution of skilled movements (22,38). Monkeys and cats with little qualitative change in limb movement following bilateral pyramidectomy show a complete loss of distal extremity movement (5) after the additional destruction of the rubrospinal tracts bilaterally (22,38). These experiments in addition to the somatotopically arranged cortico-rubral projections and the similarity in corticospinal and rubrospinal terminations suggest a functional organization to Kuypers (38). He advocates the classification of descending systems into a medial system (reticulospinal, vestibulospinal, tectospinal, and interstitiospinal) controlling postural tonus and a lateral system made up of the corticospinal and rubrospinal which is the control of fine, coordinated movement.

Isolated lesions of the RN appear to produce relatively unimportant postural changes (53). In the cat, mild extensor hypertonus is observed while in the monkey the most prevalent symptom is hypokinesis and some hypotonicity (9,34,53,58). Both animals demonstrate various symptoms of cerebellar lesions such as incoordination, ataxia, and overstepping. Massion (53) suggests that the cerebellar-type symptoms that appear when the RN is destroyed is probably due to a simultaneous lesion of the neocerebellar fibers. Also the fact that the neocerebellar system is more developed in the macaque than in the cat may explain the relative hypotonicity observed in these animals following RN lesioning.

Prior to the development of motor neuron reflex modulation and intracellular recording techniques, electrical stimulation of the RN gave limited results. In a review of the literature prior to 1932, Ingram and Ranson (34) remarked that "electrical stimulation of the red nucleus in animals with intact brains does not produce any noticeable reaction".

Although some workers have demonstrated a "tegmental response" (flexion of the anterior ipsilateral limb, extension of the anterior contralateral limb, and alternating flexion and extension of both the posterior limbs) it is generally believed that this results from stimulation of the surrounding midbrain reticular formation. Pompeiano (62) however, by using decerebrate preparation without chemical anesthesia was able to demonstrate active flexion in the contralateral fore- and hindlimbs when the stimulating electrode was placed in the caudal half of the RN. When the electrode was moved more ventrally, stimulation resulted in fore- and hindlimb flexion contralaterally, while a placement in the ventral region of the RN gave flexion of the contralateral hindlimb only. These important findings suggested that the rubrospinal tract exerted a somatotopically organized effect on the flexor musculature of the limbs.

More sophisticated experiments tend to support Pompeiano's (62) hypothesis that the rubrospinal influences predominate in the direction of flexor excitation and extensor inhibition. Thulin (78) found increased activity in the nerve to the anterior tibialis muscle during acute stimulation of the RN of the cat in contrast to an absence of activation in the medial gastrocnemius nerve during stimulation. However, the results which he obtained from monosynaptic testing methods were somewhat complex and may be "subjected to interpretive difficulties". He found that the monosynaptic response from anterior tibial nerve stimulation was facilitated by stimulation just dorsal to the RN, inhibited by a more ventral stimulation in the RN and alternately facilitated and inhibited by stimulation just caudal to the RN. He was using repetitive stimulation at 60/sec. as his conditioning stimulus and reasoned that the somewhat complex results which he obtained might be due to occlusion

at the segmental level or activation of local interneurons impinging on other mesencephalic centers. Thulin (78) also demonstrated a longer latency response on the ventral root discharge with single stimuli which he attributed to a tegmento-bulbar relay. He suggested that the oscillatory response he obtained with repetitive stimulation between the RN and substantia nigra may be due to combined action of the rubrospinal and the reticulo-tegmento-bulbar channels.

Utilizing intracellular recording techniques as well as monosynaptic modulation, Sasaki et al. (71) demonstrated the rubrospinal tract to exert facilitory effects on the flexor motoneurons and simultaneous inhibitory effects on the extensor motoneurons of the hindlimb of the cat. The EPSP's recorded in flexor motoneurons had a latency of 9 msec. while the IPSP's of the extensor motoneurons had a latency of about 11 msec.. Since then other workers have demonstrated essentially the same response with a preponderance of EPSP's in flexor motoneurons and IPSP's in antagonistic extensor motoneurons; influences were always on the contralateral motoneuron populations (30,37,71,72). This functional reciprocity between flexor and extensor motoneurons was not always observed and both groups exhibited some EPSP's and IPSP's mixed, but 80% of the extensor motoneurons exhibited inhibition and 90% of the flexor motoneurons exhibited EPSP's. The preponderance of evidence indicates at least a bisynaptic influence from the rubrospinal tract although a few isolated cells exhibited EPSP's with latencies between 3 and 8 msec. (30,37,72), thus indicating the possibility of some monosynaptic connections between the rubrospinal tract and the alpha motoneuron populations being studied. The usual EPSP latency appears to be around 8 to 9 msec.; the range of latencies recorded however, may vary from 7 to 19.5 msec.

(37).

The maximum monosynaptic focal potentials from RN stimulation were found by Hongo (30) to be in Rexeds laminae VI and VII, as one would expect from anatomical data (56). Maximum focal potentials of interneurons monosynaptically activated from both flexor reflex afferent (FRA) and low threshold muscle and cutaneous afferents appeared in the intermediate nucleus in Rexeds Layer VI (56). Thus, it would appear as though the rubrospinal tract could utilize interneurons in Layer VI common to polysynaptic reflex activity as well as those interneurons in Layer VII which were activated from the rubrospinal tract but not from primary afferents. Hongo (30) believes the latter case to be the most predominant.

Evidence has been presented which indicates that the rubrospinal tract produces presynaptic inhibition of spinal cord afferents, but the class of afferents are not identified (72). Other evidence indicates that an inhibition of the pathway from the FRA is rather common (although facilitation was observed in a few cases). This inhibition however can not be entirely due to primary afferent depolarization since it occurs before onset of the dorsal root potential that can be evoked from the RN (30).

Appleberg and Kosary (3,4) demonstrated that the rubrospinal system exerts control over gamma motoneurons as well as alpha motoneurons. Recording activity elicited in ventral root fusimotor fibers from RN stimulation, they found that there is a tendency for facilitory effects to be obtained from electrode positions ventrally in the nucleus whereas inhibitory effects predominate from electrode positions dorsally in the RN. Recordings from the dorsal root filaments containing single

afferents from muscle spindles showed that flexor spindles could be facilitated from the ventral part of the RN. Thus it appears that the RN exerts a combined effect on alpha and gamma motor systems which tends to elicit flexion of the limbs. Appleberg and Kosary (3,4) conclude that the gamma is more sensitive than the alpha excitation, but the alpha excitation is not dependent on the gamma since it is still present after section of the dorsal roots.

Gassel et al. (24,26) studied the rubrospinal activity during desynchronized sleep in unrestrained cats through chronically implanted electrodes in the RN. They found that during bursts of rapid eye movement which characterizes the desynchronized phase of sleep, a phasic enhancement of rubral activity occurs which is similar to that observed in the pyramidal tract. They concluded however that the myoclonic twitches occurring during this phase of sleep must be due to other mechanisms since they are still present after electrolytic lesion of the RN and pyramidal tract and also since evoked flexor response from repetitive stimulation of the RN is depressed throughout desynchronized sleep.

Other experiments utilizing chronic electrodes in the unrestrained animal indicate that the rubral neurons increase in activity during voluntary movement of the head or limbs (15). Stimulation of the RN by radio control for 5 seconds every minute for an hour (day and night) evoked a "reliable sequence of behavior" in unrestrained monkeys which included bipedal locomotion, climbing, vocalization and social interactions. During periods of sleep (or chlorpromazine) stimulations produced only a small head movement. It is somewhat difficult to evaluate this type of experiment since in normal conditions, local excitatory states may require the cooperation of other cerebral areas for the

performance of this type of behavior (17).

In addition to its action on the alpha and gamma motor systems of the spinal cord, the RN may exert a facilitory influence on the transmission from the primary afferents to the ventral spinocerebellar tract. Magni and Oscarsson (50) demonstrated that such a response elicited from the anterior sygmoid gyrus of the cortex depends upon the relay pathway in the brainstem. Since this pathway appears to be located in the dorsolateral part of the lateral funiculus and on account of the relatively short latency observed, the authors suggest that the pathway involved is the cortico-rubrospinal.

Monosynaptic Testing

The monosynaptic response has been reported several times as a means of monitoring induced excitability changes on motoneuron populations of the spinal cord (41,43,71,77,78). The implications of its use as a means of testing for the level of excitability in spinal cord motoneuron pools was reviewed by Thompson (77).

Rationale for the use of this testing procedure requires the nonconditioned test response to be: 1) relatively selective to a functionally independent neuronal pool; 2) to have a reliable degree of stability throughout the testing procedure; and 3) to respond in a graded manner to changes in neuronal excitability. The following résumé has been prepared to discuss the monosynaptic testing procedure relative to these three criteria.

Description and Specificity of the Monosynaptic Reflex

Lloyd (44) first classified the afferent fibers entering into the

spinal cord as: Group I which are the largest afferent fibers, and are to be found only among the afferent fibers arising from muscle. These fibers range from approximately 20μ to 12μ in diameter, and have been shown to arise from the annulospiral organ of muscle spindles and from Golgi tendon organs; Group II contains fibers of approximately 12μ to 6μ in diameter and Group III consists of fibers gathered about a peak at 3μ to 4μ. The last two groups are found in both muscle and cutaneous nerves. Group I fibers are the lowest threshold of these three types and thus may be excited in isolation by the simple expedient of selecting the appropriate nerve trunks for stimulation.

The central reflex arc delay resulting from Group I fiber stimulation has been shown to be in the order of .8 msec. indicating that arcs of two neurons are involved (monosynaptic). These two neuron-arc discharges were observed to occur only in ventral roots supplying the muscle from which the Group I fibers were stimulated, and were shown by Lloyd (44) to be associated with the phasic response to stretch described as the myotatic reflex. He referred to this response as a "local reflex" in contrast to the longer latency multisynaptic "flexor reflex" activity elicited from stimulation of the medium and small afferent fibers (II & III) (44).

Later work by Lloyd (45) substantiated his original conclusions that the monosynaptic activation of motoneurons from stimulation of low threshold Ia afferent fibers (from annulospiral endings of muscle spindles), is localized to functional groups of muscles in a limb. Although he initially concluded that the receptor field from which a motoneuron draws its monosynaptic innervation was homonymous (from the muscle supplied by that motoneuron), the observations of a monosynaptic facilitory

action was recognized to indicate that the field extended to include other synergistic muscles of the same joint (heteronymous). He designated these synergistically connected motor groups as a "myotatic unit" (46).

Eccles et al. (18) studied the convergence of monosynaptic excitatory afferents onto different species of alpha motoneurons in more detail. They substantiated the fact that muscles with closely related functions, i.e. synergists, always exhibit a high degree of monosynaptic interconnection. The ankle flexors, tibialis anterior and extensor digitorum longus have a close reciprocal linkage by receptor fields, with homonymous activation about three times more powerful than heteronymous. In a later study in 1962, Eccles et al. (20) attempted to utilize the remarkable degree of specificity for the monosynaptic excitatory action on alpha motoneurons for testing hypotheses relating to specificity of neuronal connections. Following cross-union of the nerves to the medial gastrocnemius and peroneus muscles in kittens 10 to 14 days old, there was a very high statistical significance for the development of new connections from the afferent fibers of the synergic lateral gastrocnemius and plantaris, to the peroneus motoneurons that had reinnervated the medial gastrocnemius muscles. This data would seem to further substantiate the tendency of monosynaptic connections to develop in a manner which provides for functional motor or "myotatic units".

Variability of the Monosynaptic Reflex

It is well known that the mean monosynaptic reflex amplitude decreases with increasing frequencies of stimulation. According to Lloyd (42) this depression occurs in two phases: 1) low-frequency depression,

which probably has a presynaptic origin, and is detectable at stimulation frequencies of 0.1 to 10 Hz; and 2) high frequency depressions which appear at frequencies above 10 Hz, and is attributed to subnormality of motoneurons.

Rudomin (69) showed that with a stimulus intensity resulting in 50% maximum response, the variance of monosynaptic reflexes increased with increasing frequencies from .3 to 8 Hz, although the difference in variance when stimulating at .3 or .7 Hz was not significant. By stimulation of Group I muscle and of low-threshold cutaneous afferents they were able to reduce the fluctuations of successive monosynaptic reflexes elicited by constant afferent volleys. The time course of this effect and its sensitivity to picrotoxin suggested that the paths leading to primary afferent depolarization (PAD) were involved in this variability reduction. They suggest therefore that interneurons ending on the Ia afferent terminals are highly correlated in their spontaneous activities and are, presumedly the main source of variability of the monosynaptic reflex. Reduction of excitability fluctuations of Ia terminals by afferent volleys could be explained by 1) an increase in conductance of the Ia afferent terminals during the produced PAD thereby reducing their input resistance and consequently the membrane potential fluctuations, and 2) the long-lasting depression that follows activation of the paths leading to PAD temporarily excluding them as variability sources.

Monosynaptic Response to Graded Stimuli

Variation in monosynaptic response to graded afferent volleys was studied by Hunt (32). He demonstrated that variation in a monosynaptic reflex response remains essentially constant above the level at which

the zone of variation reaches its full size, i.e. when the drive is sufficient to elicit at least a minimal response on every trail. Thus he concludes that over a considerable range the influence of variation on response amplitude remains constant and may be eliminated as a factor by using the mean of a series of responses. He also discussed the hypothetical possibility where synaptic drive is increased to a point where the mean response amplitude could be limited by the size of the pool being studied. As the drive becomes increased to this point, the zone of variation again becomes progressively diminished until all motoneurons of the pool discharge to every test volley regardless of excitability fluctuation. The considerations relating to modification of the Ia elicited discharge by limitation of pool size have little practical importance for only a small fraction of the pool discharges in usual tests of monosynaptic reflex responses; using maximal afferent stimuli the mean number of neurons firing is only about 22% of the total pool (S. D. 1.9). However, the influence of excitability fluctuation on submaximal afferent stimuli can have considerable significance for analysis of monosynaptic excitatory action if the synaptic drive was insufficient to allow for a response in those neurons where the excitability fluctuation is in a direction of decreased responsiveness. He concluded that once the zone of variation is complete, mean response amplitude may be considered to provide a direct measure of the degree of monosynaptic excitation over a considerable range of pool response.

From this brief review of literature on the monosynaptic response, it would appear that with body temperature held constant, and utilizing maximal amplitude Ia stimuli at low frequencies, the monosynaptic response can be used as an accurate readout of alpha motoneuron

excitability. The responses should however be averaged over a series of stimuli in order to allow for the variations which are present.

CHAPTER III

MATERIALS AND METHODS

Anesthesia and Maintenance of the Animals During an Experiment

The experiments utilized for this dissertation were conducted on 32 normal dogs of varying breed, age, size, and sex. The initial induction of anesthesia was accomplished by intravenous injection of thiamylal sodium (Surital, .02 mg/Kg), an ultra-short acting barbiturate. Following tracheal intubation and isolation of the vertebral and carotid arteries, the animal was connected to a positive pressure resuscitator and ether anesthesia was administered as required to maintain a proper level of surgical anesthesia. The ether anesthesia was continued throughout the surgical procedures until transection of the brainstem was accomplished; at this time the animal would become comatose and further chemical anesthesia was unnecessary. During the period of stimulation and recording, somatic movement was controlled by the injection of gallamine triethiodide (Flaxedil, 1 mg/Kg) at intervals of approximately forty-five minutes, as required. Recording procedures were always started not less than two hours after ether anesthesia was discontinued. Deep rectal temperature was monitored throughout the experiment and maintained at 37^{o} C or above with the aid of an electric heating pad and lamp.

Surgical Preparation for Stimulating the Midbrain

At the time of the ventral midline tracheotomy, the vertebral arteries were isolated at the thoracic inlet and a loop of #2 nylon suture was passed under them and then through a small plastic tube approximately six inches long. The carotid arteries were then ligated, thus making it possible to control blood flow to the brain by exerting tension on the nylon loops placed around the vertebral arteries. The animal was then placed on a Baltimore Instrument Company Stereotaxic Instrument where the head was rigidly fixed with ear bars and eye hooks. The scalp and temporal muscles were reflected to one side and a craniotomy performed with a hand trephine in the left parietal area just lateral to the parietal crest. The opening in the cranium was enlarged to extend over the saggital sinus with bone rongeurs. After the dura had been deflected from the surface of the exposed cortex, the midbrain was exposed by aspirating the overlying telencephalon. At the same time, the brainstem just rostral to the superior colliculus was severed and a transverse section about 3 mm wide was aspirated. The ventral portion of the osseous tentorium was then removed using a Kerrison Ronguer, and the basal area of the cerebellum subsequently aspirated through this opening. The extent of the cerebellectomy and brainstem transection was determined after the experiment by gross examination of the brain (Figure 1).

Typically it required 1½ minutes to expose the midbrain and to make the brainstem transection by aspiration; during this time the vertebral arteries were occluded to control hemorrhage and to insure a good field of vision. The vertebral arteries were released for 2 minutes, and then occluded for another 1½ minutes during the opening of the tentorium.

The cerebellectomy was normally achieved with the vertebral arteries released. After the cerebellectomy, a small roll from a section of gauze pad was inserted into the opening created above the midbrain and the veterbral arteries were occluded once again for one minute in order to help establish clots and to control hemorrhage as much as possible. This "gauze roll" was removed one to two hours later in order to insert the midbrain stimulating electrode. By this time the field was usually reasonably free from hemorrhage.

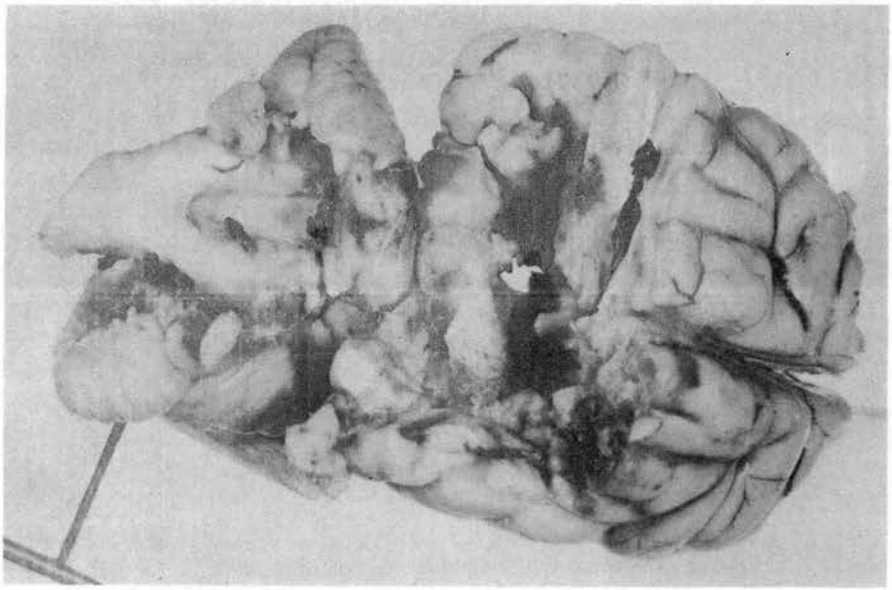


Figure 1. Photograph of Brain Illustrating Exposure of the Midbrain, Precollicular Transection, and Cerebellectomy. (A portion of the left occipital cortex was removed in order to better expose the precollicular transection.)

Citrated whole blood was administered to the animals through a cannulated cephalic vein at the rate of approximately 1 to 2 drops per second. The administration of blood was initiated at the beginning of surgery and continued as needed to maintain normal blood pressure and help prevent hemorrhagic shock. Although blood pressure was monitored directly on an E&M Physiograph for three of the experiments, the amount of blood administered was usually determined by an estimation of blood loss, and the appearance of exposed tissues. Typically, a total of 150 to 300 cc. of blood was administered to each animal.

Surgical Preparation of the Spinal Cord and Peripheral Nerves

The lumbar spinal cord was exposed by dorsal laminectomy from about the level of the L2 vertebra to the lumbosacral junction. The dura mater was then opened and 4 small stainless steel wires were placed through holes made along its cut surface. Small alligator clamps were attached to these wires in order to pull the dura up into a hammock in which the spinal cord could rest. The exposed spinal cord was covered by a pool of warm mineral oil. The ventral roots of L6, L7, and S1 segements were cut to be later placed on recording electrodes, care being taken to leave the corresponding dorsal roots intact.

The caudal end of the animal was immobilized and prepared in the same manner as described by Thompson (77):

> The spinous process of the vertebra rostral to the laminectomy was clamped in a spinous process clamp and fastened to the holder frame. The rear of the animal was elevated by a rod passed underneath the pelvis and fixed to the frame; complete immobilization of the rear of the animal was not essential. The animal could then be elevated from the bed of the holder so that respiratory excursions of the abdomen resulted in minimal movements of the spinal cord.
>
> Both rear limbs of the animal were fixed in clamps

applied to the tarsal region and attached to the holder frame. A skin incision approximately parallel to the long axis of the thigh was then made over the biceps femoris muscle group. This incision extended from a level about the proximal third of the thigh to just proximal to the stifle. After blunt dissection of the skin from the surface of the muscle, the fleshy belly of the biceps muscle was then divided by blunt dissection parallel to the muscle fibers to minimize hemorrhage. Minor hemorrhage was encountered with this procedure and hemostasis was again maintained with electrocoagulation.

The popliteal fossa was opened and the biceps was retracted to give a clear field. The fat pad of the fossa was removed to expose the sciatic nerve distal to its bifurcation into the tibial and peroneal nerves. The peroneal nerve was then severed near its entrance into the anterior tibialis muscle. The branches of the tibial nerve to the lateral and medial heads of the gastrocnemius muscles were severed and dissected free. The exposed nerves were covered with a pool of warm mineral oil and the dissected nerve branches were placed on bipolar silver stimulating electrodes.

Preparation of the Monopolar Midbrain Stimulating Electrode

The RN was stimulated with a monopolar electrode having an exposed tip of 100 to 400 microns (Figure 2) in 27 of the experiments reported in this dissertation. This electrode is a modification of the tungston microelectrode described by Stony *et al.* (76). A piece of number 4 steel piano wire ("Swedish steel" - .013 dec. diam.) was electrolytically tapered as described by Nastuk (54) in a solution of 34 pts. H_2SO_4, 42 pts. H_3PO_4, and 24 pts. H_2O. 10 v AC are applied between the wire and a carbon reference electrode immerged in this solution; a gentle taper may be obtained over approximately the wire's last 2 centimeters by slowly pulling it in and out of the solution. An abrupt point can then be etched on the wire by repeatedly touching its extreme tip to the surface of the solution. The wire is cut at approximately 14 cm. from its tip and slid inside a piece of pyrex glass tubing (i.d. = 1 mm.) about 20 cm. long. The glass tubing is then placed in a Und. Lab. Inc. glass pipette

puller in a manner such that the tip of the wire is approximately 1 cm. above the heater coil. As the glass is heated, it is pulled onto the tapered end of the steel wire. After cooling, the glass can be carefully broken off just above the etched tip where it is no longer adhered to the steel wire.

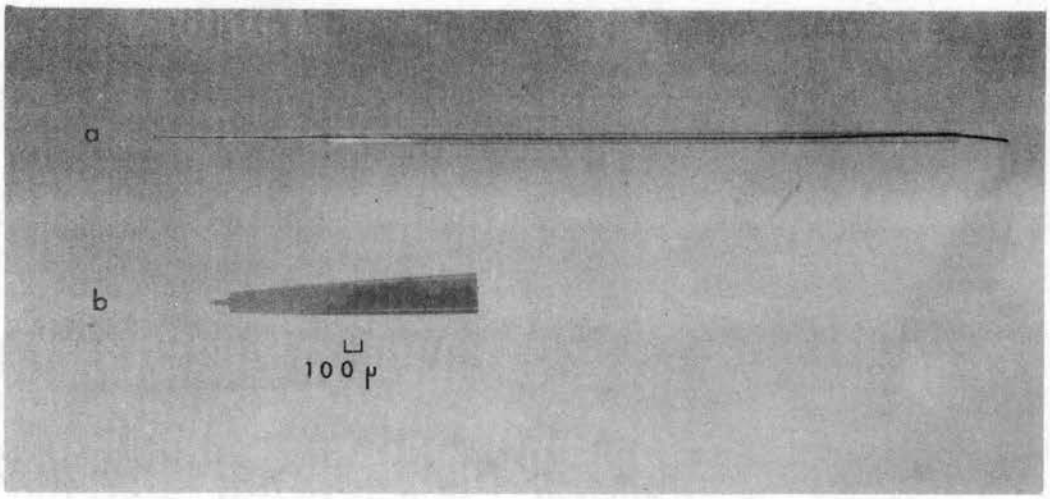


Figure 2. Midbrain Stimulating-Recording Electrode. (a) Gross appearance of glass-insulated steel wire electrode. (b) Photomicrograph of the electrode tip.

The opposite end of the glass from the tip is then filed and broken so that approximately 1 cm. of wire is exposed for contact with the recording or stimulating leads. This end of the glass is rigidly fastened to the wire by applying small amounts of dental cement or insl-x enamel. The exposed tip of the electrode may be made smaller if desired by electrolytic etching under microscopic observation.

This electrode offers the advantages of being relatively quick to make, having low resistance, and having sufficient rigidity for accurate

placement. However, the tip diameter can not be controlled to the degree necessary for research requiring no tissue damage and accurate tip dimensions.

Locating the Electrode in the Red Nucleus

Due to the variation in skull size and shape on the dogs used as experimental animals, Horsley and Clark coordinates, as worked out for beagle type dogs by Lim et al. (40), were not adaptable for accurate placement. Therefore placement of the electrode into the RN was accomplished with visual coordinates over the superior colliculus similar to the technique of Thulin (78). The Baltimore Instrument Company electrode manipulator was adjusted to 0^{o} for both the saggital and transverse angles, and the tip of the electrode was placed in a position approximately 2½ mm. lateral to the midline and between the caudal ¼ and ½ of the exposed superior colliculus. The electrode was then lowered slowly into the midbrain until maximum cord dorsum and antidromic impulses could be recorded (see Recording). This usually occurred at approximately 11 to 12 mm. of depth (Figure 3), and correlated well with histological identification of the RN.

Verification of electrode placement by histological examination of the brainstem was accomplished for most of the experiments reported in this dissertation. Following an experiment, the animal was perfused with 10% buffered formalin; subsequently, the brain was removed and placed in additional solution for a least 48 hours. Following this formalin fixation, the rostral 2/3 of the midbrain was quick frozen by wrapping it in a plastic sack and dipping it into a solution of 80% ethanol which had been cooled with dry ice. This frozen tissue was then

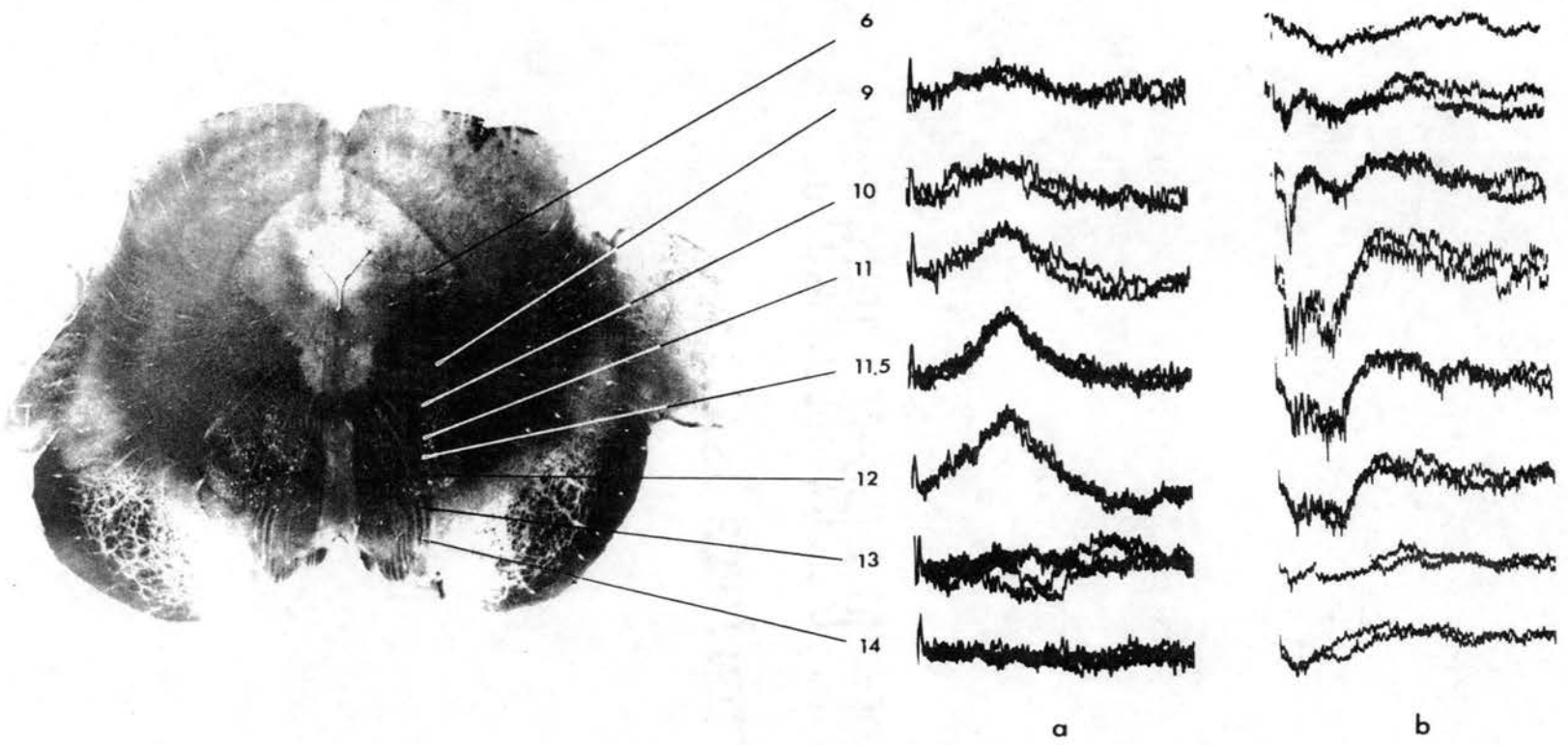


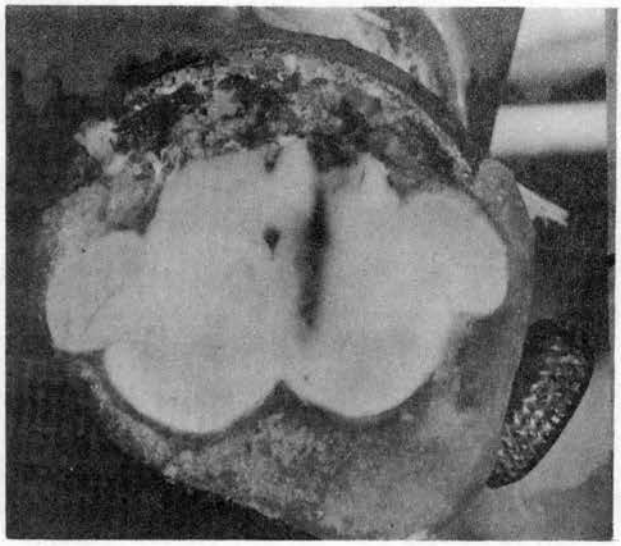
Figure 3. Electrical Activity Associated With Vertical Placement of the Midbrain Electrode. (a) Cord dorsum potentials evoked from RN stimulation and recorded from the dorsolateral surface of the spinal cord. (b) Antidromic potentials evoked from spinal cord stimulation and recorded from the RN. (Upward deflection denotes negativity in both (a) and (b). Depth of placement is indicated to the left of the above tracings in mm. below superior colliculus surface.)

transversely sectioned in an International Equipment Company Model CTD Cryostat at a thickness of 48 microns.

Depending upon the vascularity interrupted by the electrode, its tract could frequently be seen grossly on the freshly exposed surface of the midbrain during the sectioning process (Figure 4). At times however, the electrode tract could not be easily traced other than at its point of entry into the brainstem; placement of the tip could only be extrapolated from this point with knowledge of the stimulating depth utilized for the recording of data. Once the area of the electrode position was determined, slides were prepared for microscopic examination under low power in order to more precisely determine the full extent of the RN in this area. Although luxol fast blue stain was used on these sections in early experiments, it was determined that unstained sections demonstrated brainstem structures in adequate detail for the purposes of this research problem. The unstained sections were photographed with a Leitz bellows camera for future observation. An approximation of the relative caudal to rostral position of the electrode could be made by maintaining a count of the turns required on the microtome (16 microns/turn) from the point where the RN is first grossly visible to the electrode tract. An attempt was made in utilizing this data to place future electrodes in the caudal ½ of the nucleus ruber.

Stimulation

Bipolar silver wire electrodes were used for stimulation of the peroneal and gastrocnemius nerves. Square wave monophasic pulses were provided by a Grass S-4 stimulator applied through an SIU-4 isolation unit. Stimulus duration was set at 10 microseconds and a stimulus



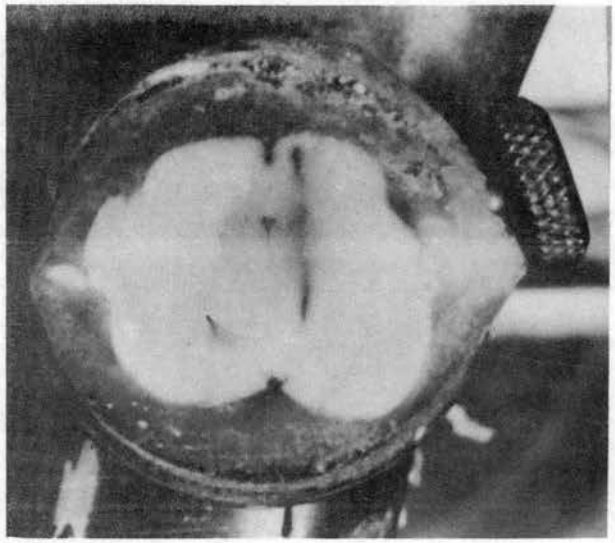


Figure 4. Photograph of two Separate Preparations as They Appear Grossly on the Microtome Mounting Block. (Note the readily visible appearance of the electrode tract.)

intensity was used which gave a near maximal monosynaptic discharge (.5 - 6 volts). In cases where multisynaptic activity was modulated, the stimulus duration was generally about .5 - 2 milliseconds, at an intensity of 2 - 10 volts.

Stimulation of the RN was accomplished with a monopolar, glass insulated, steel electrode having an exposed tip of 100 to 400 microns (see electrode preparation). A square wave monophasic pulse of .3 msec. duration was provided by a Grass SD-5 stimulator at intensities (usually 120 - 400μa) sufficient to give a maximal deflection of the cord dorsum potential (see Recording).

Synchronization pulses were taken from the variable delay circuit of the S-4 stimulator used for the peripheral nerve stimulation, and used to trigger the SD-5 stimulation of the midbrain. Thus, the interval between the RN stimulus and the test nerve stimulus could be read from the delay stimulus dial on the front of the S-4 stimulator. These dial readings are accurate within $\pm$ 5% (manufacturer's specifications).

Recording

Descending tract activity evoked from stimulation of the midbrain was recorded as cord dorsum potentials by a monopolar silver ball electrode placed on the surface of the spinal cord just adjacent to the dorsal roots of the lumbosacral enlargement. Activity was recorded from this electrode relative to a reference electrode clamped to exposed muscle of the back. This recording was useful in helping to electrically locate the midbrain stimulating electrode in the RN as well as being a monitor of rubrospinal tract activity throughout the duration of the experiment.

Antidromic activity elicited from spinal cord stimulation was utilized as a more precise means of locating the midbrain electrode in the RN once approximate positioning had been determined for low threshold cord dorsum recordings. The midbrain electrode was used in reference to a muscle electrode to record antidromic activity in the midbrain evoked by stimulation of the lumbar spinal cord by the cord dorsum electrode. The recording of antidromic wave activity and elicited spikes from surrounding neurons was assumed to indicate proper placement of the electrode in the RN.

Activity from the ventral roots of L6, L7, and S1 was recorded from a bipolar silver wire electrode. Spike activity from these electrodes elicited by brainstem stimulation was referred to as ventral root discharge. Short latency monosynaptic and longer latency multisynaptic responses were elicited from peripheral nerve stimulation and utilized as a "test reflex" to read out the excitability of appropriate alpha motoneuron pools.

All recordings were amplified by a Grass P-5 preamplifier for display on a dual beam Tektronix 565 oscilloscope. This preamplifier is capable of amplifying 35, 700, or 28 K times with 10 steps of 3 db/step attenuation in each range. Its half-amplitude frequency response is variable in 5 steps within the low range from .1 to 35 cps, and within the high range from .1 to 30 KHz. Responses for this study were generally recorded with the 28 K amplification range and a band pass from 35 to 2 KHz.

Further amplification of the evoked potentials was provided by a 3A3 dual trace differential amplifier on the Tektronix 565 oscilloscope as required to give an adequate signal for recording purposes. The dual

trace amplifier allowed the cord dorsum to be recorded simultaneously with the ventral root discharge or with a time base generated by a Tektronix RM-181 time-mark generator.

Voltage calibration of the recorded responses was made using the internal calibrator of the Grass P-5 preamplifier which gives a square wave calibration signal variable in 12 steps from 10 microvolts to 50 millivolts. The GI (active lead) is made negative with this signal, thus allowing for determination of lead polarity as well as signal amplitude.

The following procedures for testing alpha motoneuron excitability are described primarily by Thompson (77). Before testing the influences of RN stimulation on motoneuron populations, a control test reflex was established by stimulating the desired peripheral nerve and recording the monosynaptic discharge (or multisynaptic) from the appropriate ventral root. Twenty superimposed oscilloscopic sweeps of this discharge at the rate of 1.3/sec. were recorded on Polaroid film (ASA 3,000) to give an average discharge amplitude. By utilizing the delay circuit and beam brightener of the Tektronix 565 oscilloscope it was possible to isolate for display the response being studied, thereby allowing up to eight monosynaptic records to be recorded on the same frame (Figure 5).

Once a control record of an unconditioned monosynaptic discharge was established, stimulation of the test nerve was preceded at variable intervals by stimulation of the RN. This was accomplished by adjusting the delay on the S-4 stimulation of the peripheral nerve at varying intervals from 0 to 80 msec. after its trigger impulse to the S-5 stimulation of the RN. Twenty superimposed sweeps at 1.3/sec. of the conditioned discharge was recorded at each interval.

The test discharges were audio monitored by a Grass AM-3 audio

monitor which was connected to the second output connector of the P-5 preamplifier. This made it possible to count the number of discharges recorded on film without requiring direct visual inspection during the time of exposure.

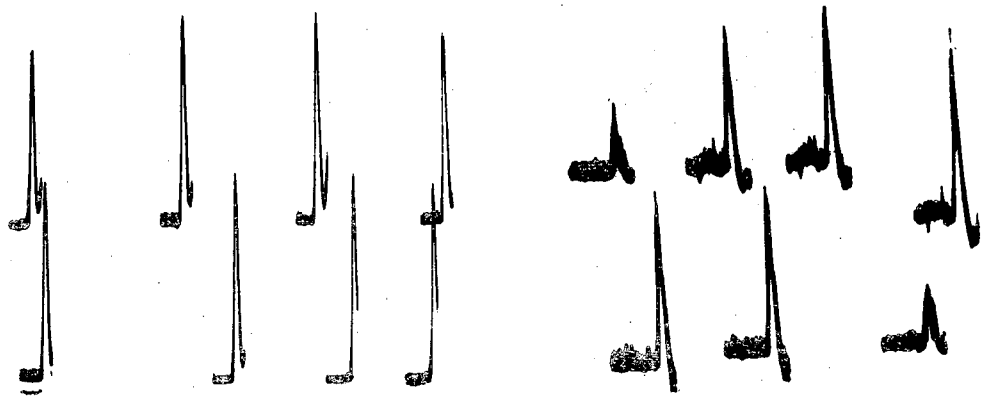


Figure 5. Two Examples of Monosynaptic Testing. (First and last tracings for each example equals unconditioned response.)

With the SD-5 stimulator for stimulation of the RN delayed at 3 msec., the S-4 stimulator delay circuit was varied at 2 msec. intervals from 3 to 17 msec., at 5 msec. intervals from 20 to 40 msec., and at 10 msec. intervals from 40 to 80 msec.. Each 4th record was taken as an unconditioned control in order to minimize recording variations. The average amplitude of the discharge at each interval (as indicated by the brightness of the superimposed sweeps) was measured by hand in divisions of 1/60 inch, using drafting dividers and an engineering rule. The resulting measurements were recorded as percent of control, and plotted on

a graph versus the time interval between stimulation of the RN and stimulation of the peripheral nerve.

Surgical Preparation for Stimulating the Ventral-Lateral Surface of the Medulla

Five of the animals utilized for the collection of conduction velocity data were stimulated on the ventral-lateral surface of the medulla, thus stimulating the rubrospinal tract directly as it passes through the lower brainstem. Stimuli to these animals were applied through bipolar silver ball electrodes with approximately 2 mm. interelectrode spacing. Arrival of the evoked impulses at the lumbosacral enlargement was recorded through a monopolar, silver ball, cord dorsum electrode.

The ventral surface of the medulla was exposed by the same technique as was described by Thompson (77) in his studies of the pyramidal system.

> The ventral midline tracheotomy incision was extended rostrally; skin and fascia were dissected away by blunt dissection to expose the anterior throidean vein which was ligated and transected. The larynx, pharynx, trachea and esophagus were reflected laterally by blunt dissection to expose the hypoglossal nerve and hyoid apparatus. The hypoglossal nerve was severed and the lingual artery was ligated and transected. The hyoid apparatus was disarticulated and blunt dissection was continued to the level of the longus capitus muscles. Hemostasis was maintained throughout this and subsequent surgical procedures by the use of electrocoagulation in addition to more conventional techniques.
>
> The longus capitus muscles were transected with an electroscalpel at the level of the atlanto-occipital articulation and reflected forward to their origin. Excision of the longus capitus muscles at their origin was completed by the use of the electroscalpel. The exposed surface of the basioccipital bone was then removed with rongeurs from the level of the bullae and caudally to the level of the occipital articular surface. The basioccipital bone was then removed with rongeurs from the level of the bullae to the articular surface of the occiput. The lateral extent of the opening was carried to a line just lateral to the medial extent of the tympanic bullae. A flap of dura mater was then removed to expose the ventral surface of the medulla from the corpus trapezoideum to just rostral to the pyramidal decussation.

Following this exposure, a steel microdissection needle was passed into the medulla at the level of the corpus trapezoideum and moved laterally and dorsally to transect the brainstem. The pyramids were then transected by use of the same microdissection needle at a point just rostral to the pyramidal decussation and caudal to the area of stimulation. All lesions were later checked by gross examination of transverse brainstem sections.

CHAPTER IV

RESULTS

Conduction in the Rubrospinal Tract

Electrical acitivity from stimulation of the midbrain and ventrolateral medullary surface was measured from the dorsolateral surface of lumbo-sacral enlargement of the spinal cord with a monopolar silver ball electrode. The resulting cord dorsum potentials were always maximal on the side contralateral to the midbrain stimulation and ipsilateral to the ventrolateral medulla stimulation. Maximal negative peak amplitudes varying from 20 - 40μv could be recorded from any point in front of or on the rostral portion of the lumbo-sacral enlargement, but the activity was diminished to nondetectable levels when the electrode was placed caudally to the lumbo-sacral enlargement (Figure 6).

The shape of the cord dorsum potential was essentially the same when elicited from stimulation of either the midbrain or the ventrolateral surface of the medulla. It consisted of a small, often indiscernable positive (3 msec. latency) wave followed by a large negative wave which frequently (8 out of 24) contained two components: a short latency ($5.159 \pm .170$), Table X in Appendix, small amplitude component and a longer latency (8.92 ± 1.05 msec.) larger amplitude component (Figure 7). The cord dorsum potential resulting from ventrolateral medulla stimulation frequently had a third longer latency component and its activity was extended over a longer period of time.

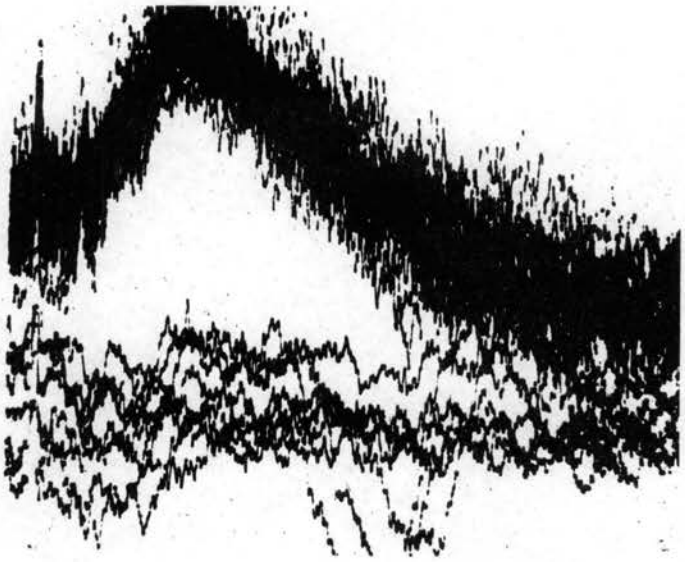


Figure 6. Cord Dorsum Potentials Evoked From the RN and Recorded From the Anterior Portion of the Lumbo-sacral Enlargement (Top) and a Point ½ cm. Caudal to the Lumbo-sacral Enlargement (Bottom). (Each tracing represents 10 superimposed oscilloscopic sweeps)

Antidromic activity evoked from spinal cord stimulation was recorded from the midbrain electrode in the area of the RN. This recording has the form of a positive potential (40 - 90μv) which, like the cord dorsum, often has two peaks, both of which were followed by negative spike activity superimposed upon the positive wave form (Figure 8). The first peak has a latency of $5.187 \pm .294$, Table X, Appendix, therefore corresponding closely to the cord dorsum positive peak (or onset of negative direction ascent). Table I shows a comparison between latencies of the cord dorsum and antidromic potentials recorded from the same series of experiments.

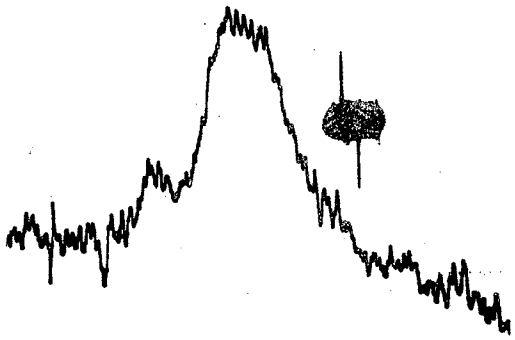


Figure 7. Cord Dorsum Potential Evoked From RN Stimulation and Recorded From the Dorsal Surface of the Lumbosacral Enlargement. (Upward deflection denotes negativity. Cal. = 10μv.)

Figure 8. Antidromic Activity Evoked From the Spinal Cord and Recorded From the RN. (Upward deflection denotes negativity. Cal. = 20μv.)

TABLE I

COMPARISON OF ANTIDROMIC POTENTIAL LATENCIES WITH CORD DORSUM POTENTIAL LATENCIES

Antidromic Latency (pos.)	Cord Dorsum Latency (pos.)	Antidromic Latency (Peak of Pos. and Spike Activity	Cord Dorsum Latency (Peak of Pos. and Beginning of Negative Ascent)
4.0	3.0	6.2	5.5
3.0	3.0	5.0	5.2
3.0	4.3	6.0	6.2
3.0	3.3	6.2	6.0
3.0	3.0	5.0	4.0
2.5	3.2	4.5	4.8
3.0	3.0	4.5	5.0
___	___	5.5	5.5
Mean: 3.07±.167	3.257±.178	5.362±.250	5.275±.244
		Correlation Coefficient .730 ± .165	

The author concluded, in agreement with Thompson (77), that the peak of the cord dorsum positive potential (when no positive potential was present, the beginning of the negative portion) corresponds approximately with the arrival of descending activity from the brain. This is in turn associated with the first peak of the positive phase of the RN antidromically evoked activity, and the subsequent negative spikes which appear at the same time.

Figure 3 illustrates the area with which evoked activity (both cord dorsum and antidromic) may be elicited or recorded within a vertical axis passing through the RN. Where precise identification of the stimulating point was possible upon histological examination, the point of lowest threshold and maximal response was always near the caudal ventral portion of the RN. Threshold for a cord dorsum response at this point was generally around 60 - 120μa (.5 - 1.4v) at .2 msec. duration; maximal response was typically obtained with stimulus intensities from 360 - 450μa (around 4 - 5 v). This somewhat specific response area would seem to indicate that most of the activity is a result of the rubrospinal tract rather than other direct or polysynaptic pathways (tegmentobulborspinal) which do not directly involve the RN.

To further reinforce the evidence for associating cord dorsum potentials with rubrospinal tract activity, an experiment was designed in which the brainstem was selectively sectioned at a level below the stimulating point and just caudal to the pons. As can be seen in Figure 9, complete ipsilateral hemisection along with section of the upper contralateral quadrant of the brainstem, left the cord dorsum relatively unchanged in amplitude and configuration. Upon completion of this transection, i.e. transection of the lower contralateral quadrant, containing the crossed rubrospinal tract (9,29,38,53,56,64,78), the cord dorsum activity was almost completely removed. Although the transections obviously were not extensive enough to completely prevent active conduction or electrotonic spread of activity across them, the relative decrease of activity which occurs after transection of the lower quadrant definitely indicates the rubrospinal tract to be the primary component of evoked cord dorsum potentials.

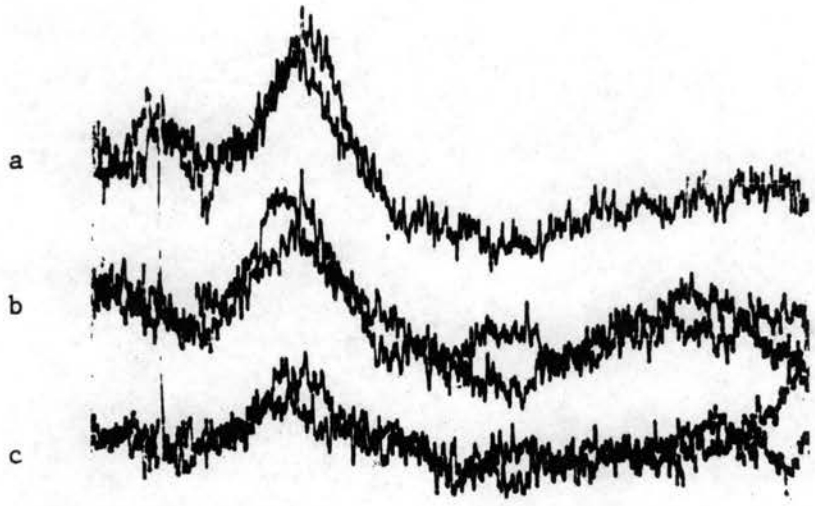


Figure 9. Cord Dorsum Potentials Evoked From RN Stimulation Following Transections of the Brainstem. (a) Control reading before transection. (b) Following incomplete transection of the medulla leaving only the contralateral ventral quadrant intact. (c) Following completion of the transection. (Above recordings each represent 3 superimposed oscilloscopic sweeps.)

Since early experiments were conducted utilizing the ventrolateral surface of the medulla for stimulation of the rubrospinal tract (thus allowing for more consistent stimulation of a large number of the descending fibers), it was felt necessary to develop experimental evidence verifying that the conduction velocities calculated from these data represent those of the rubrospinal tract. This experiment involved placement of an electrode in the RN, as well as exposing the ventrolateral medulla, and recording cord dorsum potentials elicited from both points of stimulation. In addition, antidromic activity was recorded in

the RN as elicited from stimulation of both the spinal cord and the ventrolateral surface of the medulla. After the collection of these data, the spinal cord and brain was removed, and the distances separating each of these points was measured and recorded in centimeters. From these data, conduction velocities were calculated and compared in an attempt to correlate the two stimulation points with the same fiber tract. The results of this experiment, as summarized in Table II, show that the conduction velocities are essentially the same from both stimulation points. Figure 10 shows the cord dorsum configuration as recorded while stimulating at both the RN and the ventrolateral medulla from this experiment. Note that the return to base line from the large amplitude negative phase of the cord dorsum is interrupted by a long latency negativity which is present only on that potential evoked from stimulation of the ventrolateral brainstem. Although a separate "hump" is not always discernable on the recordings, the author observed that the cord dorsum resulting from surface stimulation of the ventrolateral brainstem was frequently more dispersed than that resulting from RN stimulation. The additional long latency response would seem to indicate that spread of current to other slower conducting or multisynaptic descending systems could be taking place with the ventrolateral medulla stimulation. The author, therefore concluded that data from these preparations could be utilized for conduction velocity calculation of the fast conducting rubrospinal system, but not for a time interval study of the rubrospinal effects on spinal cord alpha motoneurons.

Table III summarizes conduction velocity data taken from eight experiments (using both RN and ventrolateral medulla stimulation) in which the brain and spinal cord were removed and carefully measured for the

distance between the stimulation and recording points. The mean conduction velocity for the most rapid conducting fibers of the rubrospinal tract was calculated to be 85.808 $\pm$ 2.915 M/sec.

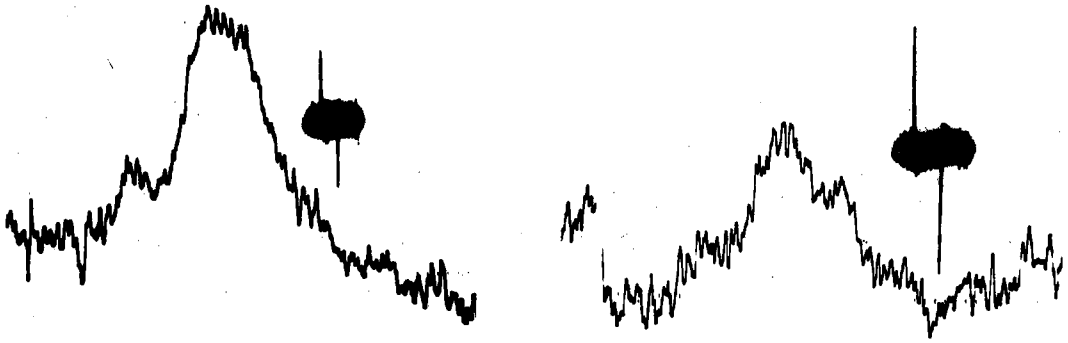
Table II

COMPARISON OF CONDUCTION VELOCITIES FROM ELECTRODE PLACEMENTS IN THE RED NUCLEUS AND ON THE VENTRAL-LATERAL SURFACE OF THE MEDULLA (ONE PREPARATION)

	Latency (msec.)	Distance (cm.)	Conduction Velocity (m/sec.)
RN to Spinal Cord	*5.5	47.8	86.9
Brainstem to Spinal Cord	*5.0	44.1	88.2
Spinal Cord to RN	**5.5	47.8	86.9
Brainstem to RN	** .4	3.7	92.5

*Cord dorsum latency (peak of pos. or beginning of rise toward negative).

**Antidromic latency (initial maximum pos. or beginning of spike activity).



a b

Figure 10. Cord Dorsum Potential Evoked From Stimulation of: (a) Red Nucleus (Cal. = 10μv) and (b) Ventrolateral Medulla (Cal. = 20μv).

TABLE III

CONDUCTION IN THE RUBROSPINAL TRACT OF THE DOG CALCULATED FROM CORD DORSUM POTENTIALS

Conduction Distance (cm.)	Reversal Latency (msec.)	Conduction Velocity (M/sec.)
39.8	4.3	92.55
37.4	4.35	86.97
47.8	5.5	86.9
35.0	4.2	83.3
36.5	4.7	77.66
33.6	4.5	74.6
39.5	5.2	75.96
41.35	4.25	97.29
		Mean: 85.808±2.915

Effects of Rubrospinal Tract Volleys on Spinal Cord Motoneuron Populations

A ventral root discharge was not normally elicited from stimulation of the RN in the midbrain; this may possibly be due to a degree of depression in the spinal cord from surgical hemorrhage, and/or the fact that a portion of the rubrospinal fibers are not fired or are injured during placement of the electrode. Single pulse, long duration (2 msec.) stimuli of 6 - 12v applied to the ventrolateral surface of the medulla (with pyramids transected) did however evoke an ipsilateral ventral root discharge (Figure 11). Table IV summarizes the latencies of these discharges and the corresponding cord dorsum potentials from the same animals. The mean latency between the arrival of the rubrospinal tract volleys and the discharge of alpha motoneurons is $2.59 \pm .178$. This latency difference, along with the long duration stimulus necessary for the discharge (allowing for repetitive firing of fibers (74)), indicates at least a disynaptic connection between the rubrospinal fibers and the alpha motoneurons. Since the beginning of the ventral root discharge has a shorter latency than the slow component of the cord dorsum potential, it is assumed that at least this initial portion of the discharge is probably a result of the short latency rubrospinal tract.

A more exact indication of rubrospinal tract influence on the contralateral alpha motoneurons was examined by means of its ability to modulate the monosynaptic response as it is elicited from the stimulation of selected peripheral nerves. Figure 12 shows the means (from 11 experiments) of the rubrospinal tract conditioned monosynaptic responses plotted as % control (nonconditioned monosynaptic response) versus the time interval between RN stimulation and stimulation of the peroneal

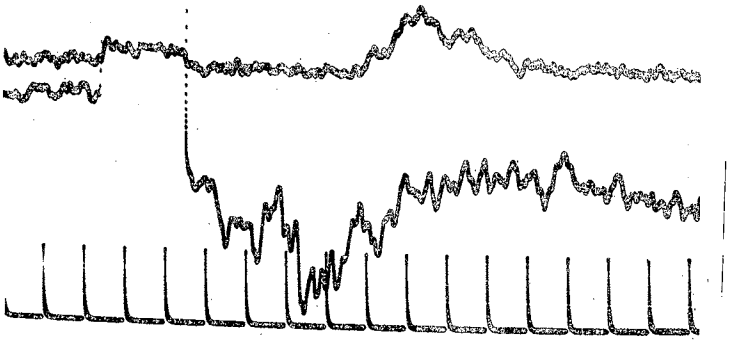


Figure 11. Simultaneous Recording of a Ventral Root Discharge (Top) and Cord Dorsum Potential (Bottom) Elicited From Stimulation of the Ventro-lateral Medulla. (Time base = 1 msec.)

TABLE IV

DISCHARGE OF LUMBAR VENTRAL ROOT FIBERS BY STIMULATION OF THE VENTRAL-LATERAL MEDULLA

Cord Dorsum Latency (msec.)	Ventral Root Discharge Latency (msec.)	Cord Dorsum Minus Ventral Root (Msec.)
4.3	6.8	2.5
4.35	7.5	3.1
4.5	6.65	2.15
4.6	6.9	2.3
5.2	8.1	2.9
		Mean: 2.59±.178

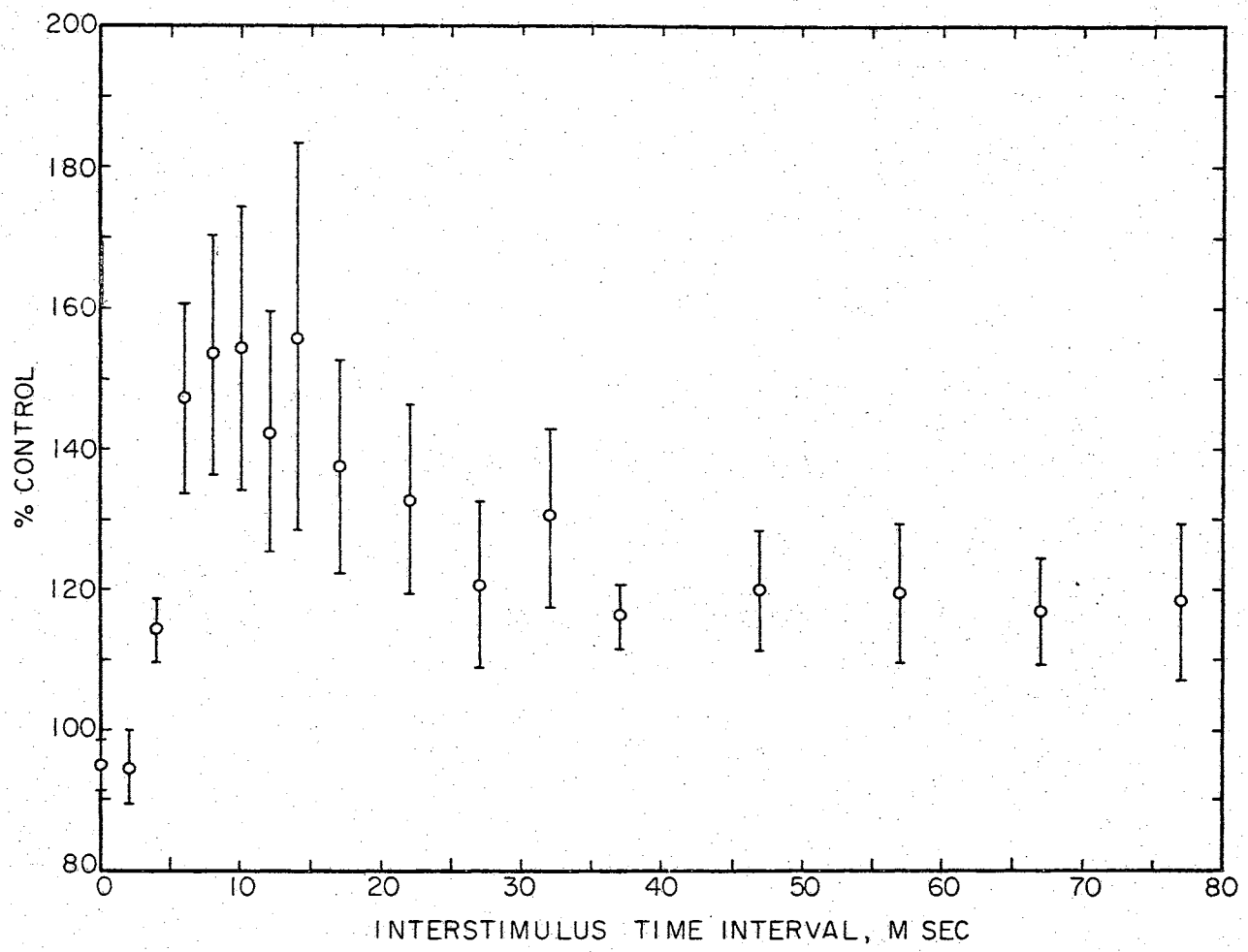


Figure 12. Time Course of Effects of Red Nucleus Stimulation on the Contralateral Peroneal Nerve Motoneuron Population. (Vertical bar represents standard error of the mean.)

nerve. The greatest degree of facilitation appeared to occur from 6 msec. to 14 msec. where the mean conditioned response varied from 147.2% to 155.8% of the unconditioned response. The paired "t-test" (74) showed facilitation from 4 msec. to 8 msec. to be significant at the $P < .05$ level. Although the mean at 14 msec. was 155.8% of the control, a statistical "t-test" shows this to be relatively non-significant due to the large amount of variation in the sample at this time interval. Time intervals from 14 msec. to 80 msec. show low degrees of significance varying about a $P < .1$ level (except at 37 msec., $P < .025$).

Rubrospinal tract modulation of the monosynaptic response elicited from stimulation of the gastrocnemius nerve of 5 dogs is plotted in Figure 13. The paired "t-test" indicates that relatively little significance should be given to the conditioned response at any level, with the possible exception of the 27 msec. and 32 msec. time intervals ($P < .05$ and $P < .1$ respectively). Examination of the individual experimental responses shows that four of the five animals from which these data were collected exhibited a mild degree of inhibition (conditioned response as low as 89.6% of control) at some point between the time intervals of 4 msec. to 17 msec. (Figure 14). Much variation occurred between experiments in the latency of this inhibition; also this inhibition was rather short lasting (approximately 4 msec. to 7 msec.) in its influences on individual preparations. This mild inhibition was followed in each of the preparations by a facilitation from 2 to 6 times the magnitude of the inhibitory response. The overlapping of these various latency facilitory and inhibitory responses resulted in a statistical mean in a facilitory direction, and in a "t"-value which could be within a normal distribution.

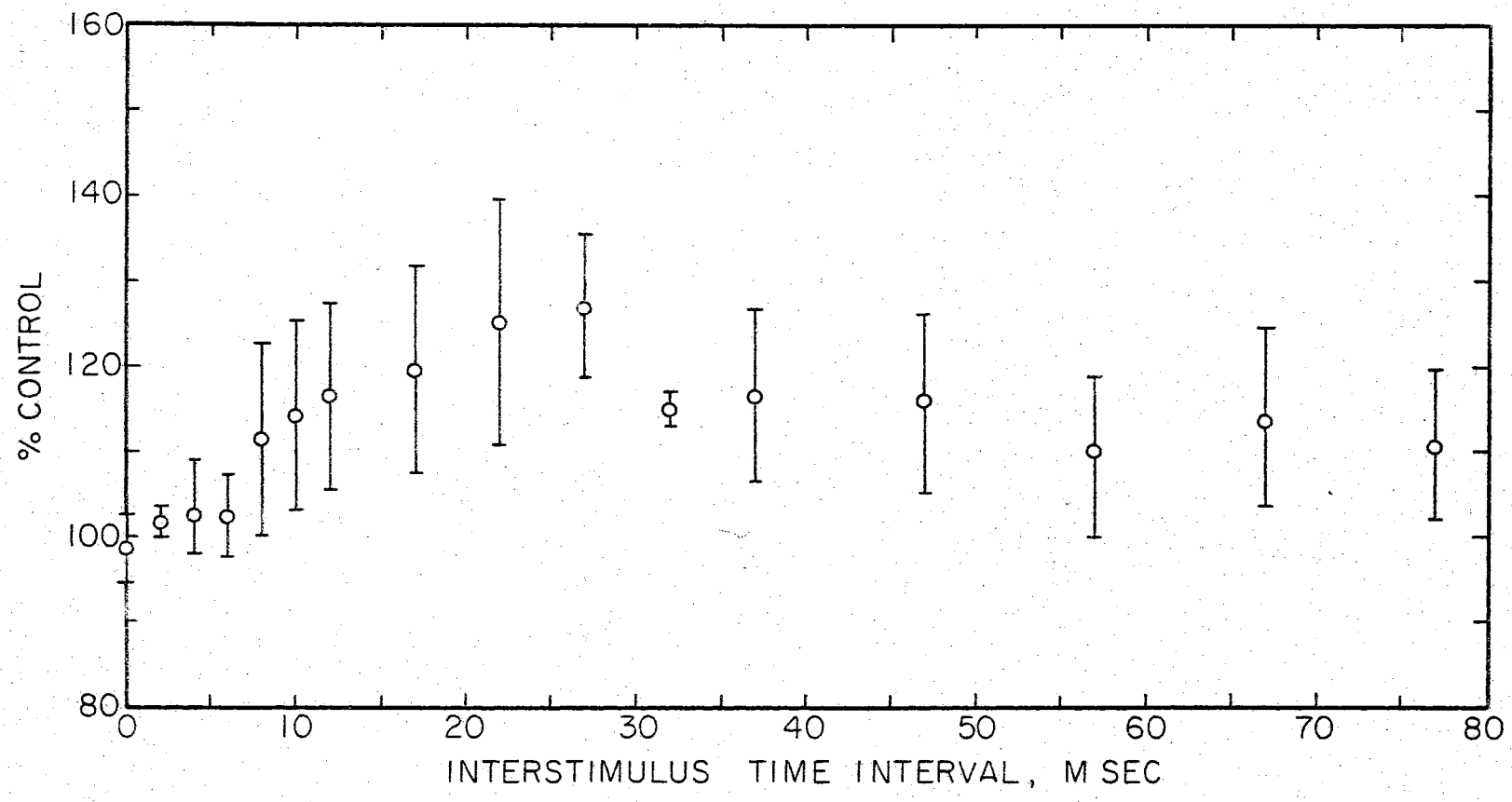


Figure 13. Time Course of Effects of Red Nucleus Stimulation on the Contralateral Gastrocnemius Nerve Motoneuron Population. (Vertical bar represents standard error of the mean.)

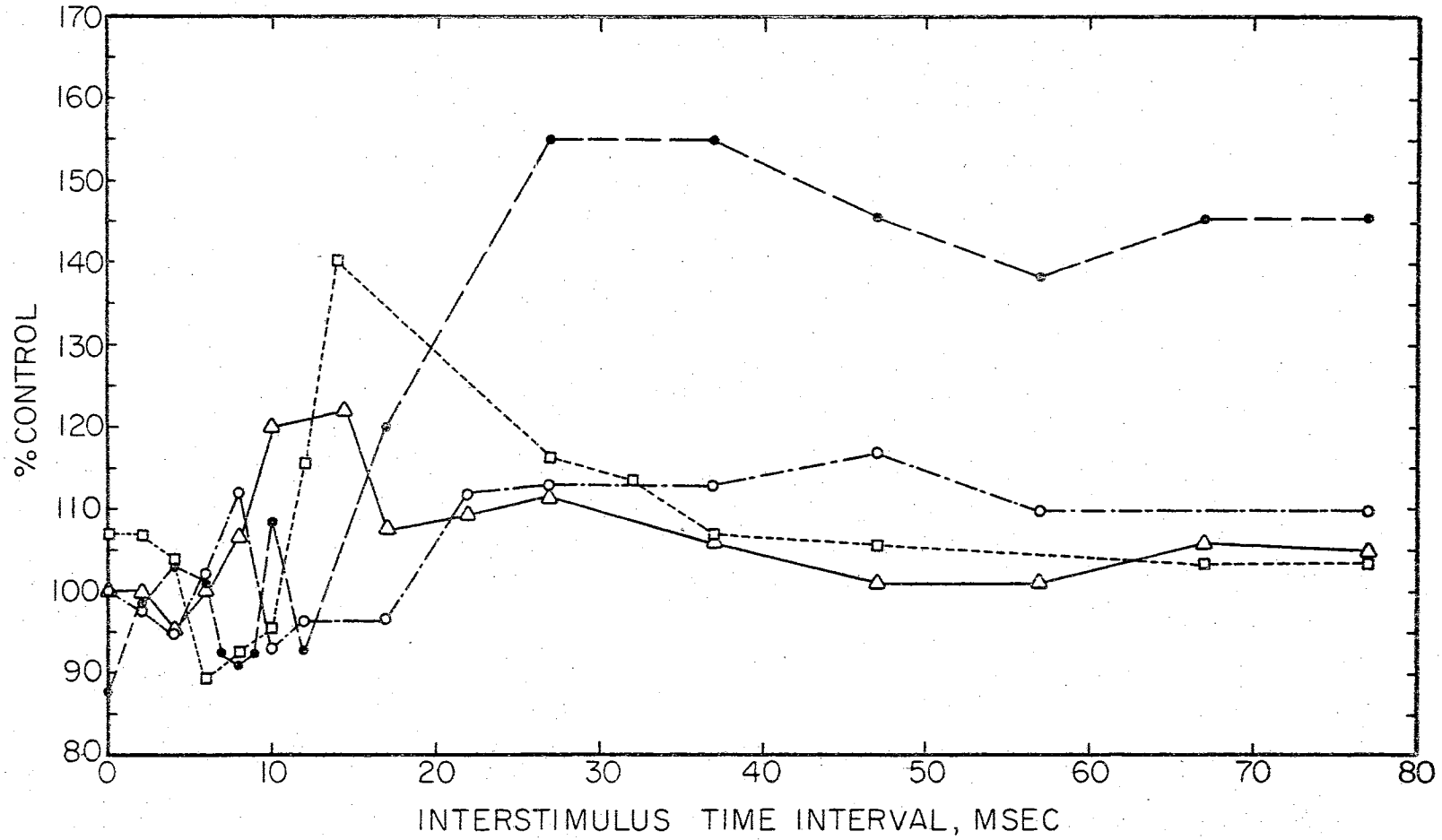


Figure 14. Time Course of Effects of Red Nucleus Stimulation on the Contralateral Gastrocnemius Nerve Motoneuron Population. Four Different Preparations Illustrated

The mean response of the gastrocnemius test reflex is plotted versus that of the peroneal nerve test reflex in Figure 15. The peroneal nerve response is statistically greater than the gastrocnemius ($P < .05$) response at the 6 msec. time interval only.

Multisynaptic activity (long latency and high threshold) (Figure 16) was elicited from stimulation of the peroneal nerve in 5 dogs and conditioned in the same manner as described for the monosynaptic response by RN stimulation. As can be seen from Figure 17, the response was extremely variable, but in general could be interpreted as strong, early facilitation, with a mean of 165% at 2 msec. and peaking at 260% with a 6 msec. time interval. The great amount of variation encountered with this procedure makes it difficult to positively interpret, and resulted in relatively non-significant "t" values at all time intervals. However there was some facilitory modulation on each of the individual experiments starting at 2 msec. with 4 out of 5 of the preparations and continuing through approximately 17 msec.

The ipsilateral activity as tested from both the gastrocnemius nerve (Figure 18) and the peroneal nerve stimulation (Figure 19), on 3 dogs each showed no statistically significant modulation when conditioned with a rubrospinal tract volley. As with the multisynaptic activity on the contralateral side of the spinal cord, this can be misleading to a certain extent; there was a slight degree of modulation in both of these preparations, but it was variable and in opposite directions. That is, both facilitation and inhibition was exhibited on different preparations for each of the two neuronal populations. Two individual examples of the modulation are plotted in Figure 20 for the peroneal nerve populations and Figure 21 for the ipsilateral gastrocnemius nerve motoneuron

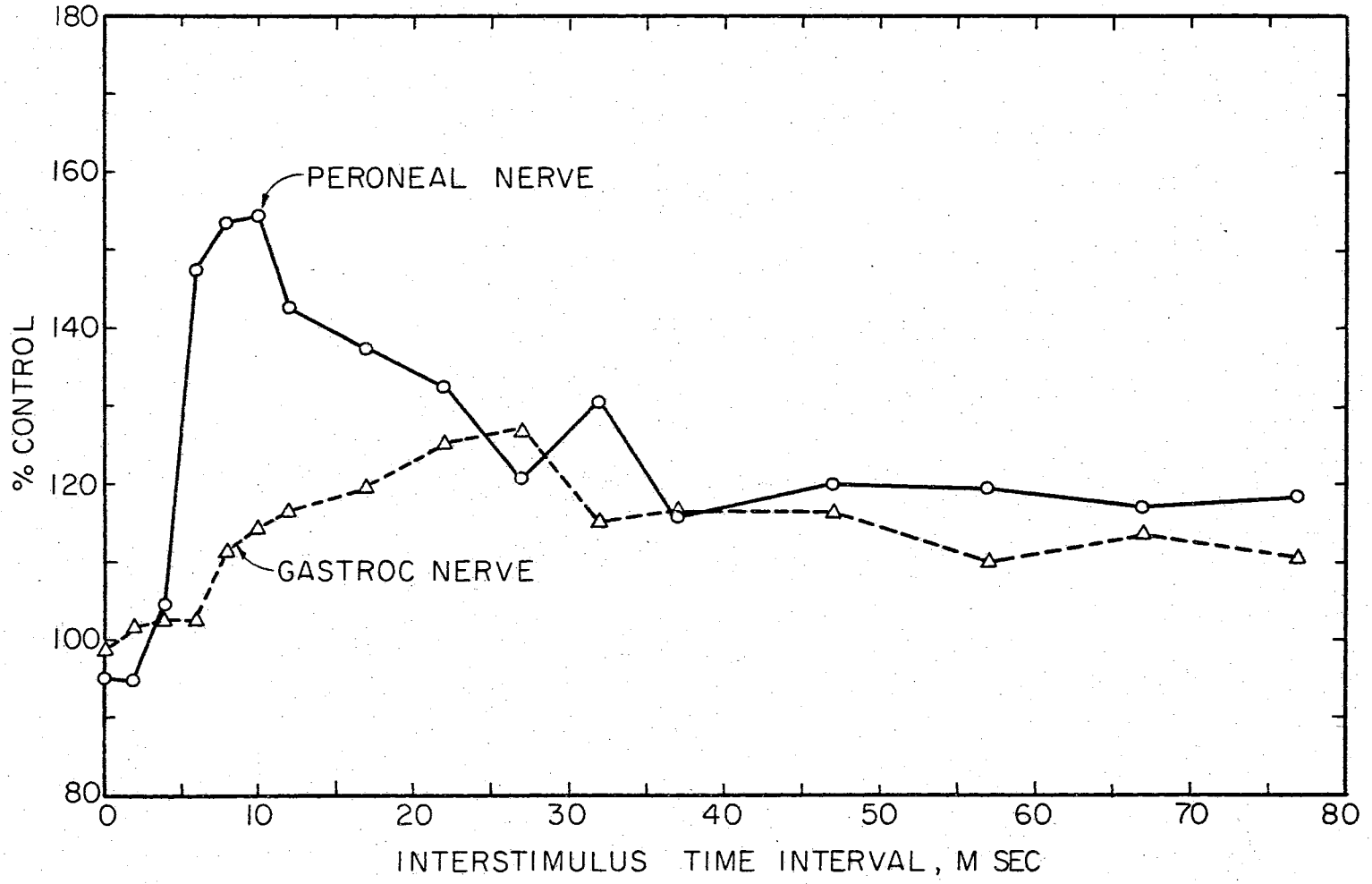


Figure 15. Comparison of Rubrospinal Effects on Contralateral Peroneal and Gastrocnemius Motoneuron Populations

populations.

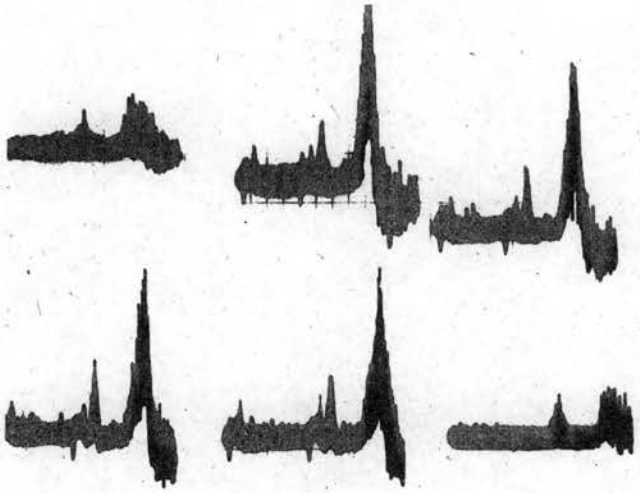


Figure 16. Example of Multisynaptic and Monosynaptic Modulation From RN Stimulation. (Time interval (msec.) between RN stimulus and peroneal nerve stimulus: Top, 0,3,4; Bottom, 5,6,0.)

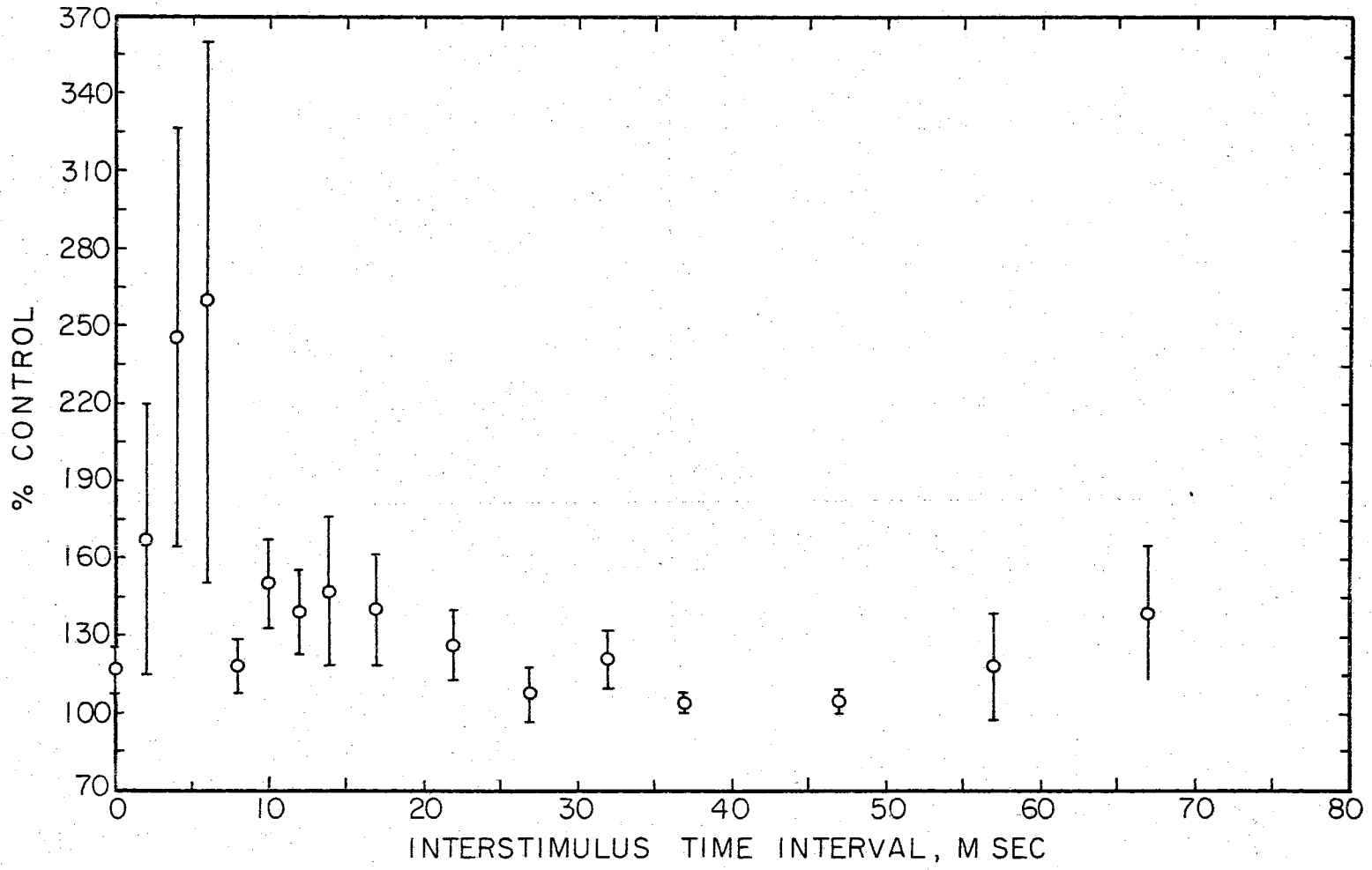


Figure 17. Time Course of Effects of Red Nucleus Stimulation on Multisynaptic Activity Elicited From Contralateral Peroneal Nerve Stimulation. (Vertical bar represents standard error of the mean.)

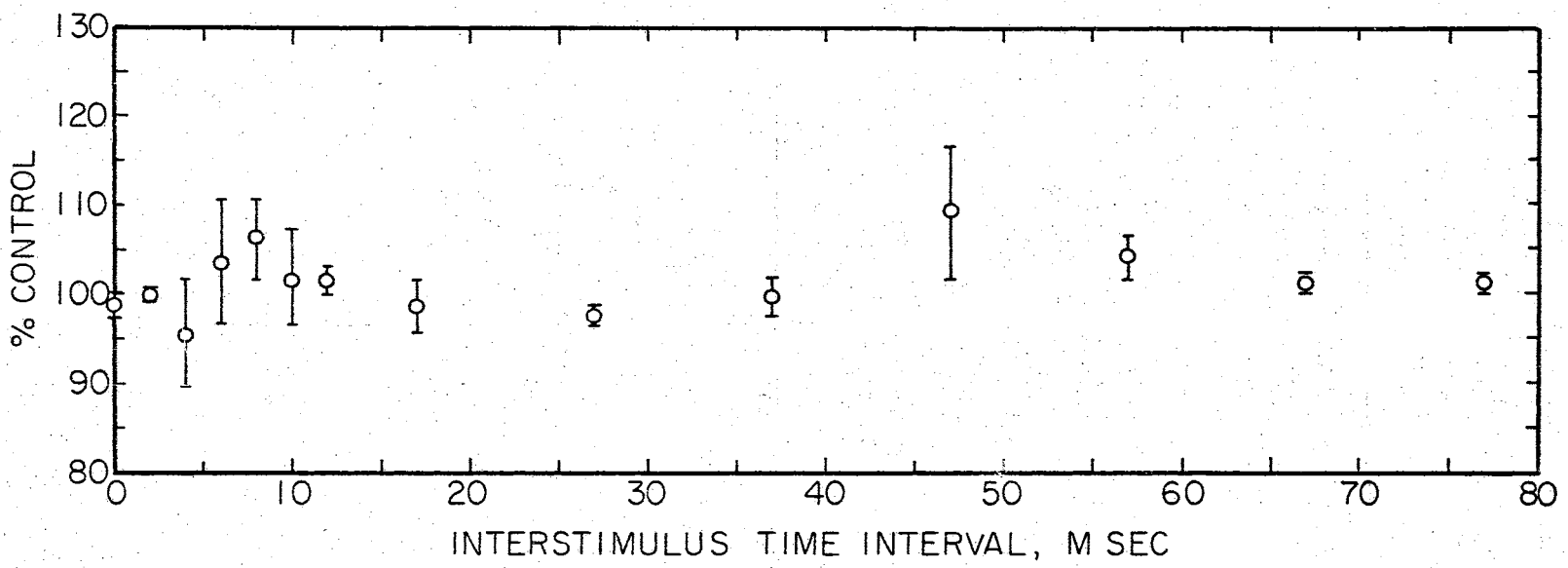


Figure 18. Time Course of Effects of Red Nucleus Stimulation on the Ipsilateral Gastrocnemius Nerve Motoneuron Population. (Vertical bar represents standard error of the mean.)

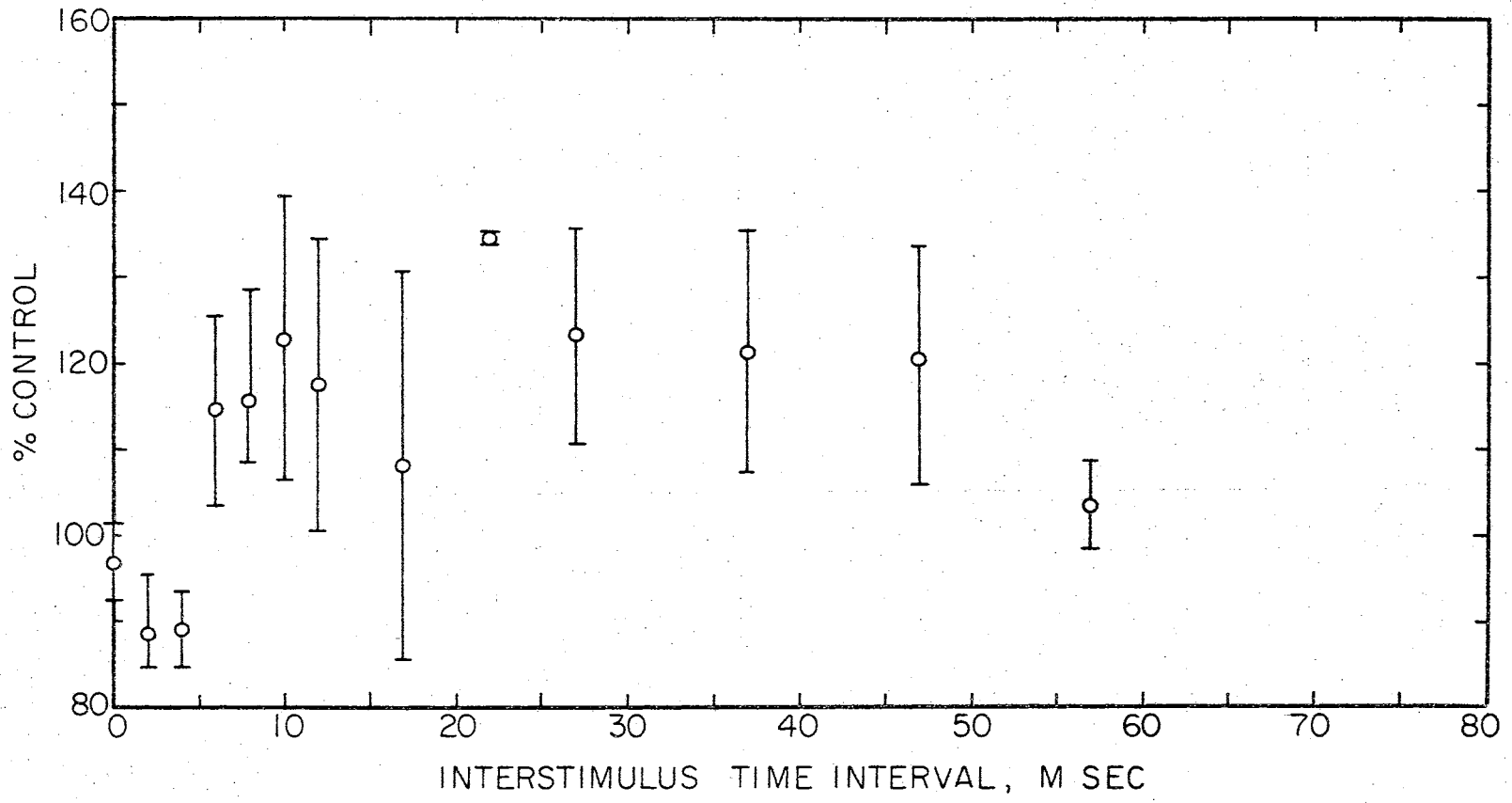


Figure 19. Time Course of Effects of Red Nucleus Stimulation on the Ipsilateral Peroneal Nerve Motoneuron Population. (Vertical bar represents standard error of the mean.)

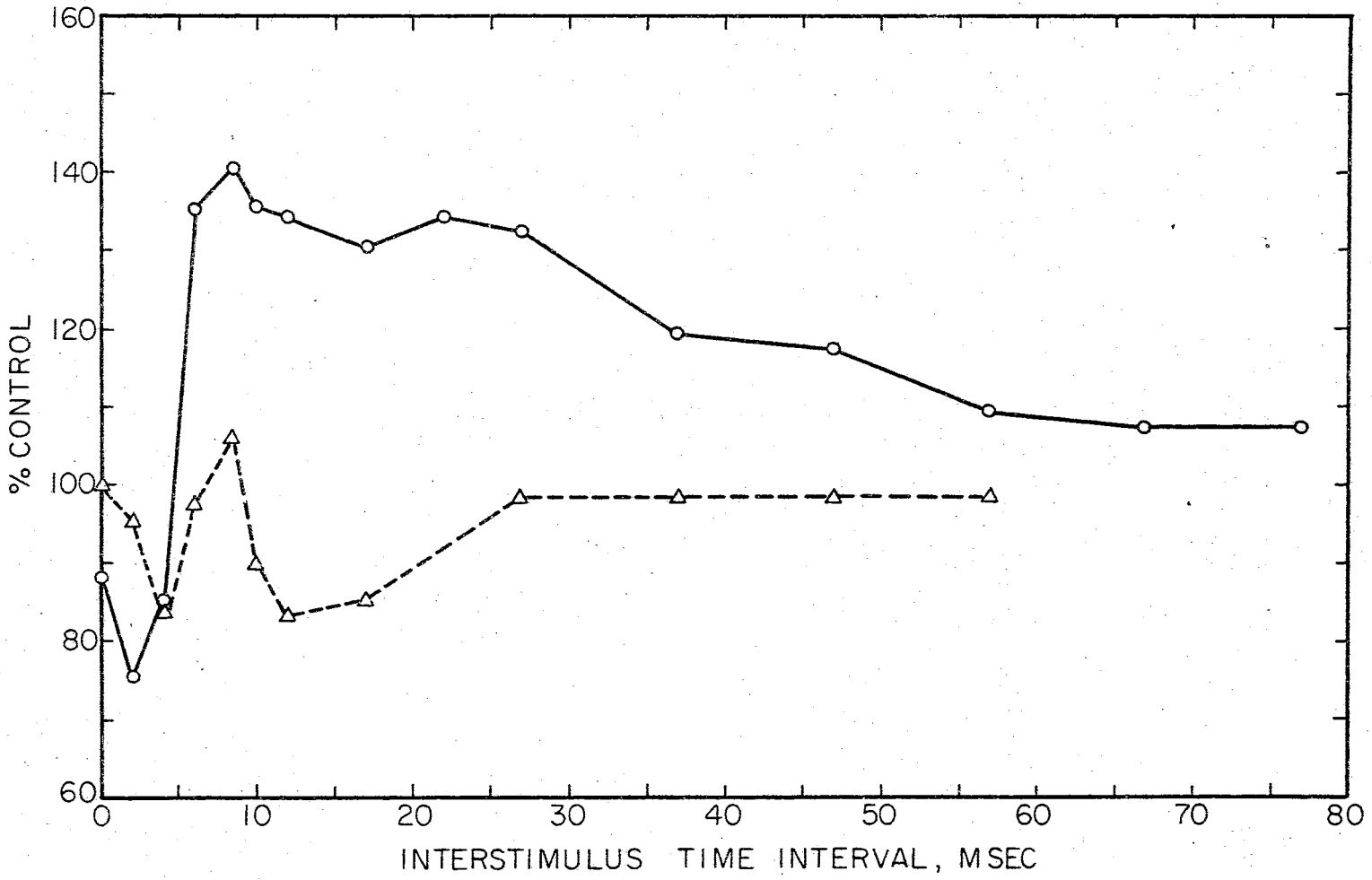


Figure 20. Time Course of Effects of Red Nucleus Stimulation on the Ipsilateral Peroneal Nerve Motoneuron Population. Two Different Preparations Illustrated

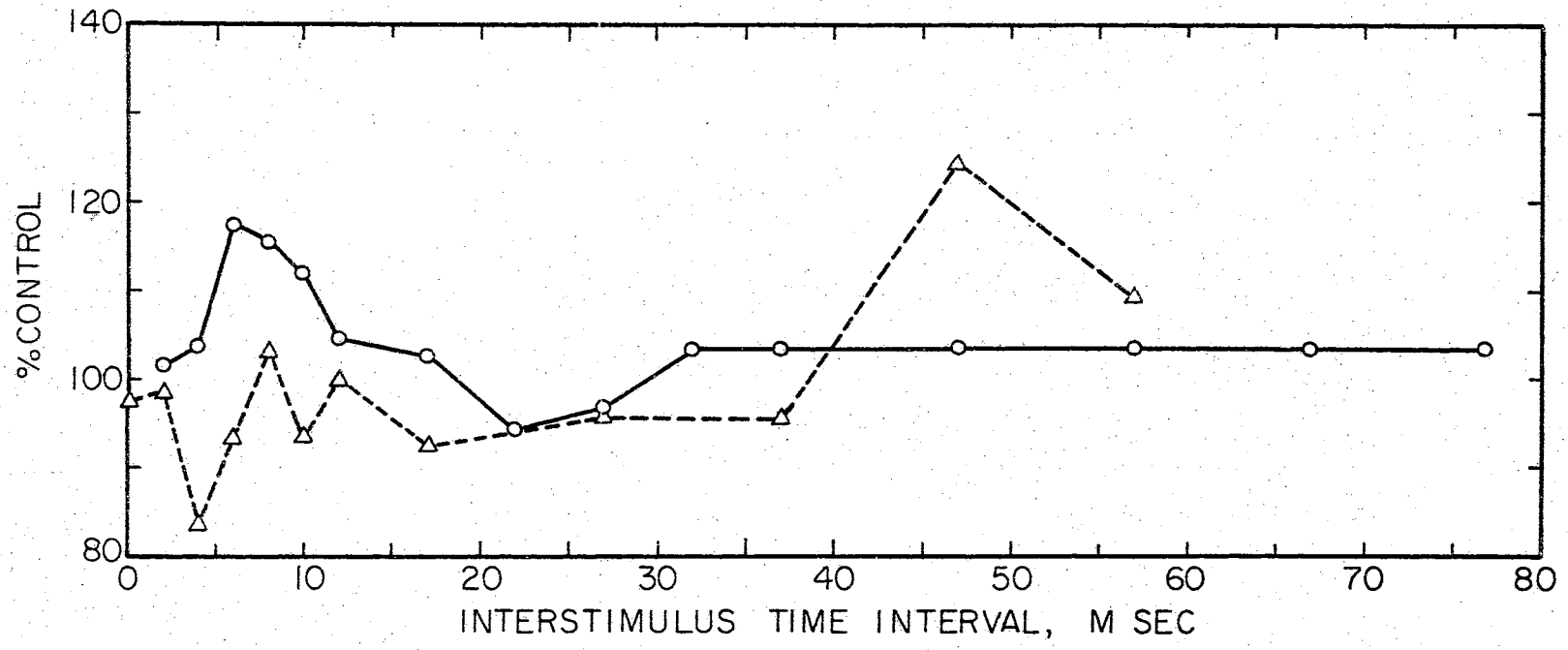


Figure 21. Time Course of Effects of Red Nucleus Stimulation on the Ipsilateral Gastrocnemius Nerve Motoneuron Population. Two Different Preparations Illustrated

CHAPTER V

DISCUSSION

Antidromic activity elicited from spinal cord stimulation and recorded from the midbrain electrode had the form of a positive wave potential. The origin of this positivity can probably be explained on the basis of impulses approaching the electrode from a distance, as would be expected from monopolar recordings in a volume conductor (68). The electrode, being located in the termination of the stimulated tract, did not experience negative activity which is characteristic of impulses moving away from the recording point. Damage of surrounding tissue may be an important factor in preventing a strong surrounding field of negativity from mass discharge of neurons in the immediate vicinity of the electrode; there are, however, prominent negative spike potentials in the recordings (although they are superimposed on positivity), indicating that some of the evoked impulses reach or pass by the electrode. Recordings very similar to this were made by Lloyd (41) while recording pyramidal tract activity from an electrode placed in the dorsal funiculus of the spinal cord.

It was assumed in this dissertation that the arrival of the first antidromic impulses to the RN corresponded with the initial peak of positivity or the negative spikes which became apparent at this time. These antidromic latencies compared well with the peak of positivity (or beginning of negative direction ascent) on the cord dorsum recordings from

the same animals (Table I). Other workers have concluded also that this is the point where the first impulses arrive at the level of the recording electrode (60,77). Phillips and Porter (60) compared recorded activity from the pyramidal tract having a similar initial positivity followed by a larger negativity, with a histogram of individually recorded fiber activity. Their compairsons showed, in agreement with the comparison to antidromic latencies in this study, that the most rapid conducting fibers arrive concurrent with the peak of the positive wave. Using the peak of the positive wave (or the point where the recording begins a negative direction) conduction velocities of the fastest fibers in the rubrospinal tract in this study were shown to be 85.808 $\pm$ 2.915. Conduction velocities calculated from the same point have been shown to be 68.9 $\pm$ 10.8 M/sec. for the pyramidal tract (77), thus indicating that the rubrospinal tract is a faster conducting system in the dog.

A fairly consistent observation of the RN antidromic recordings (elicited from spinal cord stimulation) was the appearance of two distinct and nearly equal in magnitude positive wave peaks, similar to those recorded by Tsukahara (87) from the pyramidal fiber tracts coming from the SM cortex. The large negative wave of the cord dorsum recordings also could not infrequently, (8/24) be seen to consist of two components - a short latency (5.159 $\pm$ .294) small amplitude component followed by a longer latency (8.92 $\pm$ 1.05) larger amplitude component (Figures 7, 8,9). The inconsistency of this double peak in the cord dorsum recording could possibly be related to the complexing of negative activity approaching and leaving the electrode, whereas its more defined appearance in antidromic recordings (Figure 8) is a reflection primarily of approaching tract activity only.

The appearance of these two peaks could indicate the presence of slow and fast groups of rubrospinal tract cells similar to those described in the pyramidal tract cells of the cat (87) and monkeys (23). Although a functional dual system such as this has not been hitherto described for the rubrospinal tract, it is commonly known that rubrospinal projections include both large and small diameter fibers (29,61). This makes it seem likely that, in common with the pyramidal tract system, the rubrospinal system may possess a large diameter, rapid conducting fiber system and a smaller diameter slower conducting system.

It has been shown in monkeys (23) that pyramidal tract neurons with high axonal conduction velocities tend to be active during movement and relatively inactive in the absence of movement; whereas those with low conduction velocities have regular discharge in the absence of movement and show both upward and downward deviations of discharge frequency when movement occurs. Thus pyramidal tract neurons with the largest axons seem to be phasically active in relation to movement, whereas those with smaller axons usually have tonic discharge patterns. Likewise, the spinal cord has been shown to contain small neurons with a tonic activity and larger neurons with a more phasic activity (28,36,75). Thus from a control standpoint, it would seem logical that the rubrospinal system could function more effectively with the pyramidal system in modulating the spinal cord motoneuronal system (small tonically active alpha and gamma motoneurons versus larger phasically active alpha motoneurons) if the same tonic-phasic mechanisms were made available to it. Considering the important reciprocal connections the RN has with the cerebellum, the significance of a "tonic" system which is important in muscle tone apart from a phasic system associated with rapid movements can readily be

appreciated.

A ventral root discharge could be elicited by long duration (2 msec.) single pulse stimuli of the rubrospinal tract at the ventrolateral surface of the medulla, but not from the RN electrode in the midbrain. The latency difference between this ipsilateral (the rubrospinal fibers cross above this point at the tegmental decussation) ventral root discharge and arrival of the fastest conducting fibers was $2.59 \pm .178$ (Table IV), indicating that at least disynaptic pathways to the alpha motoneurons are involved. Long duration stimuli are known to cause repetitive firing of nerve fibers (79) and thus may have allowed for temporal summation to occur in the internuncial pathways involved in the spinal cord. The fact that the same type stimulation was not normally effective in causing a ventral root discharge when applied through the midbrain electrode may simply be a result of fewer fibers being stimulated; the number of fibers fired from the RN would be variable and depend upon the rostro-caudal positioning of the electrode, and the degree of damage in the area of the electrode tract. Other investigators have utilized repetitive stimuli and were able to evoke a ventral root discharge from RN stimulation in the cat (71,78). The possibility also exists that the medullary stimulation point involves the reticular formation or the pyramidal system as well as the rubrospinal system, thus allowing for more spatial summation. However, since the beginning of the ventral root discharge has a shorter latency than the additional tract activity frequently observed on the cord dorsum potential elicited from medullary stimulus, Figures 10,11, it is assumed that at least the initial portion of the discharge is dependent on the short latency rubrospinal tract. The relatively long duration of the ventral root discharge, (3.5 msec.)

could be: a reflection of both rapid and slow conduction in the rubrospinal tract, arrival of longer latency activity from descending systems other than the rubrospinal tract, and/or graded response to the rubrospinal volley by alpha motoneurons of various sizes and thresholds.

A marked facilitation of the monosynaptic response to peroneal nerve stimulation was observed at the interstimulus intervals from 4 msec. to 12 msec. ($P < .01$ from 4 to 8 msec. and $P < .05$ from 10 to 12 msec.) (Table V). The latency observed for the test stimulus of the peroneal nerve to the monosynaptic recording was generally about 3.8 msec., thus indicating the total latency for the earliest facilitation of peroneal nerve motoneurons to be in the order of 7.8 msec.. The arrival of rubrospinal tract activity to the lumbo-sacral area of the spinal cord from RN stimulation was calculated from cord dorsum potentials to be $5.159 \pm .170$ msec.. This indicates a time interval of $2.641 \pm .170$ msec. for the transfer of rubrospinal tract activity to the alpha motoneurons of the peroneal nerve population. The above calculation corresponds well with the $2.59 \pm .178$ msec. latency for the ventral root discharge, and indicates that, in agreement with other investigators (30,37,71,72), at least a disynaptic internuncial chain is involved in the transfer of most impulses from the rubrospinal tract to the alpha motoneurons involved.

A statistical analysis of the rubrospinal tract modulation of gastrocnemius nerve monosynaptic response, showed no significant difference from the control nonconditioned monosynaptic response (Table VI). Upon examination of the individual experimental responses however (Figure 14), it can be seen that four of the five animals from which these data were collected exhibited a mild degree of inhibition at some point between

the time intervals of 4 msec. to 17 msec.. Much variation occurred between experiments in the latency of this inhibition, and it was rather short lasting (about 4 msec. to 7 msec.) in its influences on individual preparations. This mild inhibition was followed in each of the preparations by a facilitation from 2 to 6 times the magnitude of the inhibitory response; the overlapping of these various latency facilitory and inhibitory responses resulted in a statistical mean slanted towards a facilitory direction (Figure 13) and in a "t-value" which could be within a normal distribution. It may be presumptuous to give any credibility to these mild inhibitory and facilitory influences on the gastrocnemius nerve motoneuron populations, but at least they should be considered. In this context it should be pointed out that in none of the experiments involving rubrospinal conditioning of the contralateral peroneal nerve reflexes, was there an inhibitory response of time intervals greater than 4 msec.. Thus, in contrast to peroneal nerve motoneuron populations, a great deal of variation occurred in the response of gastrocnemius motoneuron populations between the time intervals of 4 msec. and 17 msec.

Results obtained from most previous investigators using the cat as an experimental animal indicate the rubrospinal system to have a definite reciprocal effect on contralateral motoneurons of the spinal cord, with facilitation of the flexor and inhibition of the extensor motoneuron pools (30,37,61,71,72). All of these investigations except those of Pompeiano and Brodal (61) were conducted on intact animals. These latter investigators used decerebrate and cerebellectomized preparations, but based their conclusions on the observation of limb movement rather than utilizing techniques to more precisely record excitability changes in

specific motoneuron pools.

The only two investigations which have studied the rubrospinal tract in decerebrate and cerebellectomized preparations (utilizing sensitive techniques which indicate excitability change in specific motoneuron pools) are those of Thulin (78), and the work presented in this dissertation. Thulin (78) failed to demonstrate a reciprocal influence on alpha motoneurons by RN stimulation, and indicated that "the heterogeneity of responsiveness of the two antagonistic types of ventral horn cells is a matter of difference of threshold rather than a difference in functional sign". The results of this thesis likewise were not conclusively reciprocal, as no significant inhibition was demonstrated in the gastrocnemius motoneuron populations. Therefore, it would seem as though the possibility of decerebration and/or cerebellectomy should be considered as a contributing factor to the variabilities obtained in this and Thulin's research from that of other workers (30,37,71,72) utilizing intact preparations. The flexion of fore- and hindlimbs observed by Pompeiano and Brodal (61) upon stimulation of the RN in decerebrate and cerebellectomized cats need not be in conflict with this hypothesis, since both Thulin and the author observed significantly greater facilitation in flexor motoneurons than in extensor motoneurons (Figure 4).

Investigators studying the pyramidal system have suggested the possibility of cortical mechanisms which could lead toward the reciprocity between flexor and extensor responses to cortical stimulation (77). Direct pyramidal stimulation has been shown by some to cause a significant increase in both flexor and extensor motoneuron excitability in the dog and cat, in contrast to surface cortical stimulation which causes excitation of flexors and inhibition of extensors (77). Thompson (77)

suggested that the differences noted between stimulation of the motor cortex and direct pyramidal stimulation could be a reflection of cortical mechanisms. If present, the same cortical mechanisms may be exerted into functional divisions of excitability in the RN.

The author's literature review shows that both anatomical (66,67) and physiological (5,81,82,83,84,85,86) evidence has been established for somatotopically organized pathways from the cortex and cerebellum to the RN. Brindley (5) was able to get somatotopically organized movement patterns in baboons with stimulation of the motor cortex after section of the pyramids. It is likely that the RN played an important part in the cortical induction of extrapyramidal pathways involved. Tsukahara (82) has shown that monosynaptic EPSP's may be recorded in the RN from stimulation of the IP nucleus and that pyramidal stimulus causes an EPSP followed by an IPSP. The EPSP's from the cortex are thought to be from slow conducting pyramidal fibers and originate from synapses on the peripheral dendrites of the recorded cells. The IPSP's from the cortex arise from somatic synapses and are produced from fast conducting pyramidal fibers, but with a delay suggesting a di- or polysynaptic pathway involving internuncial neurons in the RN (85,86). These pathways could be capable of mediating a control on RN neurons in such a manner as to help emphasize functional groupings of neuron pool excitability. An interruption of this type of control could lead to a less meaningful or "functional output upon low level stimulation of the RN".

Also, when comparing the difference between data obtained from stimulation of the RN in intact animals with those which have been decerebrated and decerebellated, one should consider the possibility of circulatory interruption to the RN during the surgical procedures involved

in a precollicular transection. The brain of the preparations utilized for this dissertation was subjected to total ischemia for three different periods of one and one-half minutes each; the carotids were ligated throughout the experiment. If one were to accept the possibility of functional mechanisms within the RN which are necessary for a simple reciprocal output, ischemic depression of these mechanisms could be the reason for the variability in the gastrocnemius motoneuron responses observed in this study (Figure 13). However, spontaneous activity could be recorded in the RN from the midbrain electrode, indicating that at least partial excitability of the neurons is present. Also, all recordings were made at least 2 hours following this acute ischemia, therefore allowing sometime for any depressed neurons to regain at least partial excitability. It therefore seems likely that depression of RN circuits is probably not a primary factor in the experiments of this study, although it may be contributory to variations caused by interruption of the cortico-rubral or cerebello-rubral pathways.

All previous investigators on rubrospinal function have been conducted on cats, whereas the studies reported herein utilize the dog as the experimental animal. Thus the lack of pronounced inhibition of gastrocnemius motoneuron populations observed in this study could be, to some extent, a reflection of species difference. The gastrocnemius complex of the cat contains a well developed tonic head, the soleus muscle (64), whereas the dog has no homologous tonic counterpart (77). Therefore the possibility exists that this species difference between the dog and the cat could account in part for the differences in results reported herein and those reporting a strong inhibition in extensor motoneurons of cats (30,37,64,72). That is, the motor nucleus of the soleus muscle

(a distinct tonic head of the triceps surae in the cat) may be dominant in the extensor inhibition elicited from RN stimulation. The lack of this muscle in the dog could then be reflected by the decrease in gastrocnemius motoneuron population inhibition observed in this study.

Another possibility that must be considered when discussing the long latency facilitation of gastrocnemius motoneuron populations (also the long latency effects on the peroneal nerve motoneuron populations), is the mediation of activity by some descending system other than the rubrospinal tract. In addition to the rubrospinal tract, the RN sends descending efferent fibers to: a) the cerebellum (6,12,13,53); b) the principal inferior olivary nucleus (53,59); and c) crossed rubrobulbar fibers which are directed to the lateral reticular nucleus (29,53). Both the inferior olivary nucleus and the lateral reticular nucleus project exclusively to the cerebellum (29,53). The experimental animals of these studies were cerebellectomized therefore making it unlikely that the variation in response could be due to any of these projections. Although direct stimulation of the reticular formation has been shown to elicit facilitory and inhibitory responses (49,51), there are no known rubrobulbar fibers ending in the brainstem reticular formation (29).

Maffei and Pompeiano (49) were able to abolish the usual flexor response (observed movement of limbs) to stimulation of the RN by sectioning the rubrospinal tract in the cervical area of the spinal cord. By sufficiently increasing their stimulus parameters, they were able to obtain a generalized increase in extensor tonus of the limbs which was especially prevalent on the side contralateral to the stimulation. In a series of experiments performed in precollicular decerebrate animals, they were able to localize a region just dorsolateral to the RN which,

upon stimulation, produced this response of increased extensor tonus. Anatomical studies of the reticular formation have been unable to show direct reticulospinal fibers originating from the mesencephalon area of the brainstem (7,27); therefore the activity from this region most likely synapses in the bulbar reticular formation in order to influence alpha motoneurons of the spinal cord. These multisynaptic pathways would necessarily have a relatively long latency. Thus, one could assume that electrotonic spread of current to this region could introduce the early variation in excitation and the long latency of gastrocnemius motoneuron populations observed in this study.

An attempt to evaluate the possibility of current spread to the dorsolateral area described by Maffei and Pompeiano (49) was conducted. Stony et al. (76), in their studies on micro-stimulation of the cortex, empirically derived a value, "$k = 1{,}292\ \mu a/mm^2$", which could be used with the expression "$i = kr^2$" relating threshold current and distance from the stimulating electrode for a given cell. Obviously, values of k will vary with differences in tissue resistance, excitability of cells being studied, and such general experimental variables as difference in level of anesthesia, blood flow, temperature, etc. The use of their k value is therefore admittedly a very crude estimate of the effective spread of current in the RN. Nevertheless, it is useful as an approximation. Using the maximum current utilized for stimulation of the RN in this study, 450μa, the solution of the above equation gives .591 mm. as the effective radius of stimulation from the exposed tip of the electrode. The largest diameter exposed tip of the stimulating electrode utilized in this study was approximately 25 - 50μ. This would give a lateral effective stimulus radius of approximately .591 + .05 = .64 mm..

The exposed length of the tip varied from 100 to 400μ, therefore giving a maximal dorsoventral effective stimulus radius of .591 + .4 = .991 mm..

The diameter of the RN in the area of stimulation was generally about 3 mm., as measured from unstained frozen sections (the 1% shrinkage occurring with this procedure (54) was considered to be of no significance for the purposes of this measurement). The above figures arrive at an effective stimulus radius of no more than 1 mm., thus indicating that if the electrode were properly placed in the middle of or in the ventral portion of the RN (as was the case with all the identifiable needle tracts), the area dorsal to the RN would not likely be affected by the stimulus. Testing of the gastrocnemius motoneuron populations was done only on those experiments exhibiting a typical facilitation of peroneal nerve populations from stimulation with the midbrain electrode. This response is typical of that observed by other workers (30,37,61,71, 72) from stimulation of the RN and thus is further indication that the electrode was positioned in the RN. However, the possibility of effective current spread to other excitatory regions of the midbrain does exist since the above calculations are only estimates, and the exact stimulating point was not always determined by histological techniques.

A comparison of the effects of rubrospinal conditioning between contralateral peroneal and gastrocnemius motoneuron populations show that the peroneal motoneuron populations are facilitated to a significantly greater extent ($P < .05$ at 5 msec. time intervals and $P < .2$ at 4, 8, and 10 msec. time intervals) from the interstimulus time intervals of 2 msec. to 10 msec. (Figure 15). In the area of 30 msec. interstimulus time interval, both populations of motoneurons experience a second area of facilitation, or at least a decrease in the rate of decline

toward control values. The means of both then remain at approximately 118% of control throughout the recorded interstimulus time intervals (80 msec.). The cause of this long latency low level facilitation could possibly be related to the long latency second peak which was observed in the cord dorsum (Figure 7) and antidromic (Figure 8) recordings of rubrospinal tract activity. The possibilities of a phasic and tonic rubrospinal system have already been discussed. Another contributory factor to this delay could be the variable length of internuncial circuits which ultimately convey rubrospinal activity to the alpha motoneurons of the spinal cord.

The degree of modulation on contralateral multisynaptic activity by rubrospinal tract volleys was variable, but always resulted in a very early (starting at 2 msec. interstimulus time interval) facilitation which showed a maximum peak at 6 msec. time interval. It then rapidly declined toward control and showed a second smaller area of facilitation which started at the 9 msec. time interval and lasted until approximately the 22 msec. time interval. The initiation of RN facilitation of multisynaptic was approximately 2 msec. before the facilitation exerted on peroneal nerve monosynaptic responses (Figure 17). This indicates that at least this initial portion of the multisynaptic modulation is caused by rubrospinal tract influences on the interneurons of the multisynaptic arc. Lloyd (41,43) made this same observation when using spinal cord reflexes to test the alpha motoneuron excitability changes in response to pyramidal stimulation.

Hongo et al. (30) observed that the rubrospinal tract caused a spatial facilitation on some pathways from low threshold cutaneous afferents, but mostly inhibition on the pathways from high threshold

flexor reflex afferents (FRA). They concluded from their study that the excitation of flexor motoneuron pools is predominantly mediated via those interneurons in Rexed's Layer VII that are activated from the rubrospinal tract but not from primary afferents. The early multisynaptic facilitation observed in this study shows that at least a portion of the internuncial neurons conveying rubrospinal tract activity to the alpha motoneurons are common with those mediating high threshold multisynaptic activity.

Stimulation of the RN resulted in latency dependent graded levels of response when utilizing the monosynaptic test as a readout of alpha motoneuron excitability (Figures 12,13,18.19,21,21). In contrast to this, modulation of multisynaptic responses resulted in a relatively abrupt pattern of strong early facilitation (Figure 17). This could indicate that the time dependent graded responses of motoneuron discharge to rubrospinal bombardment is a result of mechanisms in the spinal cord which are not directly involved in the mediation of activity to the alpha motoneurons.

Henneman et al. (28) and Somjen et al. (75) have shown that the size of a motoneuron is the chief determinant of its excitability, and that excitability dictates the order of recruitment regardless of the source of stimulus or the neural circuits which transmit it to the motoneurons. Small cells with slow conducting axons have a higher input resistance, and require weaker stimulating currents to reach threshold than do larger cells with rapid conducting axons. The spectrum of sizes and thresholds in the pool makes it an anatomically built-in grading mechanism, which responds automatically to any input and emits an appropriately "sized" output. Therefore, the recruitment, by supraspinal descending systems,

of additional motoneuron units into the discharge zone are apt to be related to the size of the individual motoneurons (28,75).

The modulation of ipsilateral motoneuronal populations was slight in most cases, and not consistent enough to be statistically significant. Individual experiments did show a slight degree of modulation on both the gastrocnemius and peroneal nerve preparations, but it was quite variable and even in opposite directions between experiments. Thompson (77) concluded that ipsilateral effects from pyramidal stimulation were probably due to intraspinal mechanisms mediated by commissural interneurons such as those which mediate crossed reflex actions from flexor reflex afferents. The same is probably true for ipsilateral rubrospinal tract effects.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A study of rubrospinal influences on selected lumbar motoneuron populations of the spinal cord was conducted for the first time in the decerebrate and decerebellate dog. A monopolar, glass insulated, steel electrode was utilized to stimulate the RN and to record antidromic rubrospinal tract activity elicited from spinal cord stimulation. Orthodromic activity was elicited from RN stimulation and recorded from the dorsolumbar surface of the spinal cord. Excitability changes induced by stimulation of the RN were detected by monitoring monosynaptic spinal cord reflexes elicited from the stimulation of selected peripheral nerves in the hindlimb of the animal.

Examination of the cord dorsum and antidromic potentials shows the appearance of two peaks, indicating the presence of both fast and slow conducting fibers in the rubrospinal tract. The possibility of a phasic and tonic system being present was discussed in light of evidence for such a functional division in the pyramidal system and in spinal cord motoneuronal pools. The presence of such a "dual" system within the rubrospinal pathways has not been hitherto described and should be investigated in future studies.

Evidence was presented which indicates the arrival of antidromically evoked spinal acitivity in the RN can be associated with the first peak of the positive potential and subsequent negative spikes which appear

at this time. Latencies determined from this point on the antidromic recordings compared well with latencies on the same preparations of the cord dorsum potential peak of positive (or beginning of negative ascent). The mean conduction velocity calculated from cord dorsum latencies indicates the fastest conducting rubrospinal fibers transmit activity at 85.808 $\pm$ 2.915 M/sec. To the knowledge of the investigator, no previous studies on conduction velocities of the rubrospinal tract are available for comparison.

The time course of rubrospinal effects on contralateral lumbar motoneuron populations, as tested with the monosynaptic reflex from the peroneal and gastrocnemius nerves, did not exhibit a purely reciprocal effect as might be expected from previous results in the cat. The peroneal nerve motoneuron populations showed a significant facilitation at an average latency indicating at least disynaptic internuncial pathways between rubrospinal fibers and the alpha motoneurons. The gastrocnemius nerve motoneuron populations, however, exhibited a varying response to rubrospinal tract stimulation rather than the inhibition one would expect from the data of previous studies. The possibility of this variation being due to an interruption of cortico-rubral or interpositorubral fibers was discussed. Further investigations of the importance of the cortex and cerebellum in determining functional divisions of excitability in the RN are indicated.

The rubrospinal modulation of multisynaptic activity typically resulted in facilitation approximately 2 msec. before the observed facilitation of peroneal nerve elicited monosynaptic activity. This indicates that at least a portion of internuncial neurons involved in transmission of rubrospinal tract influences to the alpha motoneurons is common with

those of multisynaptic reflex activity.

The ipsilateral motoneuron populations showed no statistically significant changes in excitability, although examination of the individual experiments reveals a variable (in both direction and magnitude) amount of modulation to be present. Since the rubrospinal tract is a crossed system, this ipsilateral response to RN stimulation is believed due to spinal cord mechanisms such as those mediating crossed spinal cord reflex responses.

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A P P E N D I X

TABLE V

TIME COURSE OF EFFECTS OF RED NUCLEUS STIMULATION ON CONTRALATERAL PERONEAL NERVE MOTONEURON POPULATIONS

Time Interval (msec.)	% Control	Standard Deviation	Standard Error	Vs. Control P <
0	95.0	12.2	3.8	N.S.
2	94.9	16.9	5.1	N.S.
4	114.4	14.9	4.5	.01
6	147.3	44.5	13.4	.01
8	153.5	55.0	16.6	.01
10	154.2	60.8	20.2	.05
12	142.4	48.7	17.2	.05
14	155.8	54.6	27.3	N.S.
17	137.4	43.1	15.2	.05
22	132.7	35.6	13.4	.10
27	120.7	33.2	11.7	N.S.
32	130.4	32.0	13.0	.10
37	116.1	12.3	4.6	.025
47	120.0	23.3	8.8	.10
57	119.5	23.9	9.7	N.S.
67	117.0	18.8	7.6	.10
77	118.3	27.6	11.3	N.S.

TABLE VI

TIME COURSE OF EFFECTS OF RED NUCLEUS STIMULATION ON CONTRALATERAL GASTROCNEMIUS MOTONEURON POPULATIONS

Time Interval (msec.)	% Control	Standard Deviation	Standard Error	Vs. Control P <	Vs. Peroneal P <
0	98.77	8.0	4.0	N.S.	N.S.
2	101.90	3.43	1.53	N.S.	N.S.
4	102.58	10.04	4.49	N.S.	.20
6	102.50	10.80	4.83	N.S.	.05
8	111.48	25.19	11.26	N.S.	.20
10	114.26	24.42	10.92	N.S.	.20
12	116.38	24.34	10.88	N.S.	N.S.
14					
17	119.52	24.55	12.27	N.S.	N.S.
22	125.13	24.59	14.19	N.S.	N.S.
27	126.96	18.34	8.20	.05	N.S.
32	115.00	2.82	2.00	.10	N.S.
37	116.60	22.29	9.96	N.S.	N.S.
47	116.17	21.16	10.58	N.S.	N.S.
57	110.00	18.88	9.44	N.S.	N.S.
67	113.85	21.37	10.68	N.S.	N.S.
77	110.92	19.57	8.75	N.S.	N.S.

TABLE VII

TIME COURSE OF EFFECTS OF RED NUCLEUS STIMULATION ON IPSILATERAL PERONEAL NERVE MOTONEURON POPULATIONS

Time Interval (msec.)	% Control	Standard Deviation	Standard Error
0	96.9	7.4	4.3
2	88.8	11.3	6.5
4	89.0	7.5	4.3
6	114.5	19.1	11.0
8	115.6	17.8	7.2
10	122.9	28.5	16.4
12	117.3	29.6	17.0
14			
17	108.0	31.9	22.5
22	134.4	0.3	0.0
27	123.2	22.0	12.7
32			
37	121.2	24.2	13.9
47	120.1	23.9	13.8
57	103.4	7.6	5.3

TABLE VIII

TIME COURSE OF EFFECTS OF RED NUCLEUS STIMULATION ON IPSILATERAL GASTROCNEMIUS MOTONEURON POPULATIONS

Time Interval (msec.)	% Control	Standard Deviation	Standard Error
0	98.7	1.8	1.3
2	99.9	1.2	0.7
4	95.8	10.5	6.0
6	103.7	12.5	7.2
8	106.1	8.0	4.6
10	101.7	9.4	5.4
12	101.5	2.7	1.5
14			
17	98.6	5.1	2.9
22			
27	97.4	2.2	1.2
32			
37	99.7	3.8	2.2
47	109.3	13.0	7.5
57	104.2	4.5	2.6
67	101.1	2.0	1.1
77	101.1	2.0	1.1

TABLE IX

TIME COURSE OF EFFECTS OF RED NUCLEUS STIMULATION ON CONTRALATERAL MULTISYNAPTIC ACTIVITY

Time Interval (msec.)	% Control	Standard Deviation	Standard Error	Vs. Control P <
0	116.23	15.88	9.17	N.S.
2	168.12	105.47	52.73	N.S.
4	246.0	181.7	81.2	.20
6	260.6	223.2	99.8	.20
8	118.5	21.3	10.6	.20
10	150.5	34.0	17.0	.10
12	139.2	38.6	17.2	.10
14	147.6	50.1	28.9	N.S.
17	140.5	43.2	21.6	.20
22	126.5	19.0	13.4	N.S.
27	107.5	19.7	11.3	N.S.
32	121.5	16.2	11.4	N.S.
37	104.3	7.5	4.3	N.S.
47	105.3	9.2	5.3	N.S.
57	118.7	30.0	21.2	N.S.
67	139.5	37.4	26.4	N.S.

TABLE X

CORD DORSUM AND ANTIDROMIC LATENCIES RECORDED FROM ANIMALS UTILIZED IN THIS STUDY

Cord Dorsum Latency (Peak of Pos. and/or Beginning of Negative Ascent	Antidromic Latency (Peak of Pos. and Spike Activity
4.3	6.2
4.35	5.0
4.5	6.0
4.6	6.2
6.1	4.5
5.0	5.0
6.8	4.5
5.5	4.1
5.2	Mean: 5.187±.294
6.2	
6.0	
5.0	
4.3	
5.7	
4.0	
5.5	
4.8	
6.1	
5.4	
4.8	
4.2	
Mean: 5.159±.170	

VITA

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