COMPARISON OF CUTANEOUS REFLEX PATHWAYS IN FLEXOR AND EXTENSOR MOTONEURONS BETWEEN CHRONIC SPINAL, ACUTE SPINAL AND UNLESIONED CATS

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MASTER OF SCIENCE IN PHYSIOLOGY

by

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ABSTRACT

In cats with chronic transection of the spinal cord, gentle stroking of the sole of the foot can elicit a powerful flexion reflex that normally requires a painful stimulus in the intact animal. Is this phenomenon due to removal of descending input or to a reorganization of segmental reflexes? Intracellular recordings of postsynaptic potentials (PSPs) were made in motoneurons (Mns) of 3 groups of animals: 2 acute spinal, 9 chronic spinal (transected 6 weeks previously) and 5 spinal cord intact animals. The polarity and latencies (<5ms) of the earliest postsynaptic potentials following low threshold stimulation of hindlimb cutaneous nerves were recorded in 110 hamstring motoneurons: 67 innervating knee flexor/hip extensors (PBST) and 43 innervating hip extensors (SMAB). In intact animals PBST Mns received excitatory input from most cutaneous nerves tested except for the tibial nerve, which innervates the sole of the cat foot, and consistently produced no effects. However, in acute and chronic transected cats, tibial excitatory PSPs (EPSPs) occured in 59 out of 67 PBST Mns. SMAB Mns in all animals received a variety of synaptic effects form cutaneous stimulation with tibial stimulation consistently producing inhibitory PSPs (IPSPs) in 25 out of 33 of these Mns in acute and chronic transects. Latencies of tibial EPSPs and IPSPs were not significantly different between preparations. The results indicate that hamstring flexors are more predictable in

their response to cutaneous stimulation than extensors. Acute and chronic spinal cord transection increased the incidence of EPSPs to flexors (PBST) and IPSPs to extensors (SMAB) following tibial stimulation. This pattern of PSPs would suggest that this cutaneous pathway is involved in the flexion reflex despite activation at very low stimulation strengths. The similarity of tibial effects in acute and chronic spinal animals implies that increased reflex excitability, when the sole of the foot is stimulated, is due to the removal of a tonic descending inhibition rather than a segmental reorganization of afferent input.

Introduction

The purpose of this thesis is to survey the resultant synaptic effects in hindlimb motoneurons from stimulation of low threshold cutaneous afferents in acute and chronic spinal cord transected cats as well as intact anesthetized cats. A comparison of synaptic effects in these three different preparations is based on what is known from cutaneous reflex testing in transected cats. Following acute transection the animal is initially flaccid (Ashby and McCrea 1987). After a period of approximately 1-2 weeks has elapsed, hyperactive stretch reflexes and cutaneous reflexes can be elicited (Ashby and McCrea, 1987). An example of the latter is the flexion reflex which involves flexion of the hip, knee and ankle. It has been observed that flexion of the hindlimb in a cat transected two weeks earlier can be easily elicited using gentle manipulations of the toes (Ashby and McCrea 1987). In the spinal cord intact cat this flexion behavior is thought to be used as a withdrawal mechanism but only when a noxious painful stimuli is applied to the foot. If we assume that the flexion reflex produced from low threshold stimulation in transected cats is similar to that produced as a withdrawal mechanism in intact animals, we predict that transection produces changes in low threshold cutaneous pathways.

Acute transection of the cat's spinal cord may result in a lowering of the threshold of cutaneous stimulation to produce the en masse flexion reflex, as compared to a spinal cord intact animal. However, there is an initial 1-2 week period where the flexion reflex is depressed. In the chronically transected animal (greater than 3 weeks post transection) it is easier to elicit the flexion reflex compared to the acute transected animal(within a week of transection) (Ashby and McCrea 1987). Other differences between the acute and chronic transected animals can be found in comparing flexion and extensor reflexes. The flexion reflex has been found to emerge at an earlier time post transection than extensor reflexes such as the extensor thrust elicited from the sole of the cat's foot (Sherrington 1910). Transection of the spinal cord appears to affect extensors more than flexors. Because flexors appear to have a more direct link to descending control (see Descending Control of the Flexion Reflex) than extensors, acute removal of this control has a powerful influence on expression of flexor spinal reflexes. Extensors have less reliance on descending mechanisms so that other variables may play a larger role in the expression of their reflexes. For example, sprouting of axon terminals to make new connections or to take up vacant spots left by degeneration of transected fibers maybe activated during the initial period following transection.

(Goldberg and Murray, 1983). A combination of release from descending control as well as sprouting of axon terminals may contribute to the emergence of hyperactive segmental reflexes by altering the transmission of afferent input through spinal interneurons involved in segmental reflexes. The reflex activity in the animal six weeks post transection will depend on the balance of loss of descending control versus sprouting plus other biochemical and biophysical changes (Gustaffson 1982, and Nafichiti 1980) and how each of these factors relate to flexors and extensors. Based on these assumptions, we would predict that we are more likely to see a different pattern of synaptic effects in chronic spinal animals as compared to acute spinal animals, especially with regards to flexors and extensors.

The Flexion Reflex

A clinical description of the flexion reflex, one of the first reflexes to emerge from the period of spinal shock, was first reported by Sherrington (1910). Following electrical stimulation of peripheral nerves, as well as natural skin stimulation, he found that the cat will flex the ipsilateral limb and extend the contralateral limb. Widespread flexion of the hindlimb which included the ankle, knee and hip, was termed the flexion reflex. He used the response of a muscle to stimulation as a means by which to classify muscle groups as flexors or extensors. If a muscle group contracted during the reflex it was called a flexor and if the the muscle group was inhibited ie. inactive, it was called an extensor (Sherrington 1910). No matter what area of the limb was stimulated, including flexor or extensor muscle afferents as well as joint and cutaneous afferents, contraction of flexors and inhibition of extensors was the result. Differences were found in the ease with which a certain stimuli could evoke the reflex in spinal cord transected cats. (Sherrington 1910, and Creed et al 1932) For example, a painful high threshold stimulus including muscle and cutaneous afferents was most effective in eliciting the flexion reflex in the spinal animals. However, there were exceptions in that low threshold gentle stimuli could also produce widespread limb flexion, particularly when applied to the skin of the foot of the spinal animals. Other exceptions to the high threshold effects were seen when stimuli applied to the skin of the groin, buttock and perineal regions as well as pressure of the plantar skin of the foot of the cat, resulted in excitation of extensor muscles, in spinal animals. Often a gentle stimulation of these receptive fields elicited excitation in extensors whereas a more noxious stimulus produced inhibition more typical of the flexion reflex response.

Eccles and Lundberg (1959) using intracellular recording from motoneurons in acute spinal cats anesthetized with pentobarbitone also demonstrated a common pattern of synaptic effects that correlated with Sherrington's studies (1910). They found that those muscles classified by Sherrington as flexors received excitatory postsynaptic potentials (EPSPs) from group II and group III muscle afferents and cutaneous afferents as well as joint afferents and that similar afferents produced inhibitory postsynaptic potentials (IPSPs) in muscles classified as extensors. On rare occasions it was also shown that synaptic effects opposite to that predicted by the flexion reflex could be elicited from large receptive fields following stimulation of muscle and cutaneous afferents. Because of the similarities of the synaptic effects produced by higher threshold muscle, cutaneous and joint afferents Eccles and Lundberg proposed that they be called the flexion reflex afferents (FRA) (Eccles and Lundberg 1959).

Low Threshold Cutaneous Reflexes

Differences in stimulation strength of cutaneous afferent nerves produced different reflexes in extensors. Sherrington (1910) described low threshold cutaneous pathways of the foot which resulted in excitation of extensors of the hindlimb whereas higher thresholds resulted in inhibition of extensors and excitation of flexors (the flexion reflex). Intracellular studies that examined synaptic effects from stimulation of cutaneous nerves innervating the skin of the foot (Engberg 1964) did not correlate with Sherrington's observations (1910). Low threshold electrical and natural stimulation of the central pad of the foot of the cat, resulted in excitation of the toe extensor muscles but not other hindlimb extensors. This low threshold pathway may have a specialized function in stabilizing the foot during locomotion (Engberg 1964). This pathway is not considered to be an FRA effect as it is localized to one joint of the hindlimb. In contrast to the low threshold effect, higher strength stimulation of the central pad however, did result in excitation of other hindlimb flexors and inhibition of extensor motoneurons, which correlates well with Sherrington's descriptive studies (1910). These results provided an example where a difference in stimulation strength produced opposite effects in extensors, low thresholds producing excitation and high thresholds producing inhibition. Further, low threshold effects were seen to be either NONFRA ie. localized to one joint(Engberg 1964) or similar to the flexion reflex (Sherrington 1910).

The work of (Engberg 1964) further illustrates the complexity of cutaneous afferent systems and the difficulty in isolating cutaneous reflex effects. In the same study described above, he also demonstrated that high threshold muscle and cutaneous FRA afferents from other parts of the limb elicited IPSPs in toe extensor motoneurons whereas there was as an unusually high incidence of EPSPs especially from cutaneous afferents (Engberg 1964). In the many reflex pathways available to toe extensors, cutaneous afferents appear to converge onto the excitatory pathway that also receives the specialized input from the low threshold cutaneous afferents of the foot. In other words, an FRA excitatory pathway could be in series or in parallel to the specialized excitatory pathways to toe extensors. This pattern of convergence appears to be different for the high threshold muscle and joint afferents which converge onto the inhibitory FRA pathways to the toe extensors muscles.

Earlier studies done by Hagbarth(1952) demonstrated that not only the strength of stimulation of afferents could isolate reflex pathways but that the location of receptive fields was also an issue in differentiating localized cutaneous reflexes from the flexion reflex. He found examples of excitation of extensor muscles that appears to be due to special cutaneous pathways, but related to the location of the receptive field rather than a difference between strength of stimulation. He examined monosynaptic reflexes (MSR) using cutaneous stimulation as a conditioning input, and found that stimulation of the skin overlying an extensor muscle facilitated the extensor monosynaptic reflex while the flexor monosynaptic reflexes were inhibited by skin overlying the antagonistic muscle. For example, skin overlying quadriceps (knee extensor) would inhibit monosynaptic reflexes from knee flexors, and skin overlying the ankle extensors would excite the ankle extensors. Stimulation of other areas of skin from the hindlimb would produce effects consistent with the flexion reflex, that is facilitation of the flexors and inhibition of the extensors.

Hagbarth (1952) also made two important observations involving electrical stimulation of cutaneous afferents. Firstly, he found no correlation between the specific skin areas that produced localized effects and the peripheral receptive fields of the cutaneous nerve trunks. For example, the specific skin area overlying ankle extensors that produced facilitation of the MSR did not resemble the known receptive field of the sural nerve which innervates skin of the posterior leg and part of the foot of the cat (Reighard and Jennings,1935). Secondly, he examined the effects of different kinds of adequate stimuli on monosynaptic reflexes including stimulation of nociceptive high threshold afferents, and low threshold mechanoreceptor afferents. It was found that no matter which modality was tested, the resultant effect on the MSR was similar. For example, stimulation of nocioceptive and mechanoreceptive receptors from skin overlying the ankle extensors produced excitation of the MSR from the ankle extensors. It was concluded that the best predictor of the reflex effect was related to the area of the skin stimulated rather than the type of cutaneous receptors activated. Therfore, in describing cutaneous effects using electrical stimulation of cutaneous nerve trunks one should be aware that the nerve may contain afferents from skin areas that evoke reflexes with opposing actions. For example it is possible to activate simulataneously with electrical stimulation low threshold pathways that are similar to the flexion reflex or low threshold pathways that produce effects opposite to that of the flexion reflex afferents.

Work done by Schomburg and Steffens (1986) provides further evidence that cutaneous pathways cannot be described on the basis of threshold for stimulation alone. These investigators compared the effects of electrical high threshold (5T) and natural stimulation which included light stroking as well as noxious heat, in high spinal cats. They found that in some motoneurons, mainly extensors, the electrical high threshold stimulation produced discrete membrane hyperpolarizations(IPSPs) while adequate mechanoreceptor and noxious stimulation produced membrane depolarizations and/or an increase in synaptic noise. Because the synchronous electrical stimulation of even a single cutaneous nerve contains fibers from many receptive fields an FRA effect was seen in the extensors. Natural stimulation of a specific area of skin can activate a smaller receptive field and may therfore, allow a more local effect to be seen. Taken together with the earlier studies described above, these results emphasize that cutaneous afferents cannot be grouped together only on the basis of stimulation strength to isolate a reflex pathway, especially low threshold cutaneous pathways.

As discussed above, in spinal animals gentle manipulation of the toes elicits the flexion reflex in spinal animals, whereas more noxious stimuli are needed in spinal cord intact animals. Because low threshold pathways appear to be released in spinal animals, it is important to understand that these pathways have also been shown to be controlled from supraspinal centers in isolation of the flexion reflex pathways. Hongo et al.(1969) found that stimulation of the red nucleus could facilitate transmission to motoneurons in low threshold cutaneous pathways but not those from FRA afferents. This work suggests that low threshold pathways also have a "private" descending control. Therefore, cutaneous input can be involved in either an isolated localized effect or in a more widespread FRA response depending on the convergence of descending signals and peripheral input.

Descending Control of the Flexion Reflex

What do we know about cutaneous reflexes following immediate release from descending control? In 1961 Holmquist and Lundberg examined the descending control of spinal reflex pathways using preparations with various brain stem lesions and assessing effects from natural or electrical stimulation of peripheral afferent nerves. This paper demonstrated that the control from higher centers to flexors is independent of that to extensors. A low pontine lesion released inhibitory effects from FRA afferents to both flexor and extensor motoneurons. A lesion more caudal in the neuraxis at the level of the medulla resulted in flexor excitation following stimulation of flexion reflex afferents with the inhibitory effects to extensors being unchanged. However, the possibility of exceptions to the FRA pattern were once again evident since it was noticed that extensors received EPSPs from FRA afferents in the spinal as well as the preparations with low pontine and caudal medullary lesions. Therefore for flexors, descending systems dictated the pattern of effects seen in motoneurons innervating flexors. If it was a spinal lesion, excitatory effects were seen in flexor motoneurons from FRA afferent stimulation, but if it was a low pontine lesion, inhibitory effects were observed. Extensors did not have this pattern dictated from supraspinal centers because in the spinal cat either an excitatory or inhibitory FRA pathway could be found, although the former was not as common as the latter.

Eccles and Lundberg (1959) provided evidence using intracellular recording as well as monosynaptic reflex testing, that some muscle afferent pathways became more excitable following removal of descending input. Following transection of the spinal cord both inhibitory and excitatory pathways of group Ib and group II muscle afferents produced a greater number of effects in the transected cat as compared to the decerebrate cat. The Ia inhibitory pathway was unaffected following transection. As a general rule flexor motoneurons that were excited by group II muscle afferents were also excited by cutaneous afferents stimulated electrically. Extensors also showed a consistent pattern, wherby, extensor motoneurons inhibited by group II muscle afferents were also inhibited by cutaneous afferents. Subsequent to these studies cutaneous afferents have been shown to converge onto muscle afferent pathways including Ib interneurons, group II interneurons as well as the Ia inhibitory interneuron (Hultborn 1981 for most recent review, Lundberg et al 1987 for group II effects). Following transection these muscle afferent pathways released from descending inhibition could provide a pathway of excitation for cutaneous inputs. This common convergence from group II muscle and cutaneous afferents resulting in excitation of flexors and inhibition of extensors of the spinal cat is consistent with the earlier studies describing the flexion reflex (Sherrington, 1910 ; Eccles and Lundberg 1959).

Eccles and Lundberg (1959) also demonstrated an increase in the number of effects from stimulation of Ib afferents in the transected animals as compared to the decerebrate cat. However, there was no apparent pattern of convergence similar to that of the group II afferents. For example, in a motoneuron pool that received inhibition from stimulation of Ib afferents it also received excitation from cutaneous afferents. The Ib interneuron has been studied extensively in recent years, particularly Ib pathways from ankle extensors and quadriceps onto different motoneurons of the hindlimb (Czarkowska et al 1981; Jankowska et al 1981; Jankowska et al 1983). These interneurons have been shown to receive extensive convergence from low threshold cutaneous inputs as well as high threshold FRA (Harrison and Jankowska 1985) (Lundberg, Malmgren, Schomburg 1977). Harrison and Jankowska (1985) have examined the pattern of convergence onto these interneurons and have found that there is no simple classification of how the input is organized, but that the input appears to be grouped into many different subpopulations. For example, we cannot predict the results of synaptic effects from Ib afferents based on whether a muscle is a flexor or an extensor. Because the Ib pathways are not consistent with common convergence from many afferents typical of the flexion reflex, it is hypothesized that it could be involved in the transmission of localized low threshold cutaneous pathways. This line of reasoning does not exclude other interneurons such as Ia, or group II interneurons, that also may convey low threshold cutaneous NONFRA effects.

Summary

Based on the earlier descriptive studies of Sherrington (1910) the idea of the flexion reflex, a group of afferents producing excitation of flexors and inhibition of extensors, emerged as a way to describe the organization of cutaneous reflexes. Intracellular correlates were soon described by Eccles and Lundberg (1959). Sherrington also described pathways that resulted in excitation of extensors from skin areas such as the foot, which conflicted with the flexion reflex. However, Eccles and Lundberg in comparing the effects of different lesions of the neuraxis introduced the idea of alternate FRA pathways, which they found in both flexors (received inhibition from FRA) as well as extensors (received excitation from FRA afferents). It is possible that the reflexes from skin areas innervating the foot as described by Sherrington could be alternate FRA pathways. However, as discussed earlier there are examples of specialized pathways that also excite extensors but from small specific skin areas, related to low threshold stimulation(Engberg 1964). Other examples of specialized pathways to extensors were also discussed, but these pathways were distinguished from the FRA on the basis of location of the skin receptive fields rather than stimulus strengths (Hagbarth 1954).

Historically, excitatory pathways to extensors as well as the inhibitory pathways to flexors have been termed alternative FRA pathways. (Holmquist and Lundberg 1961). The terminology is confusing in that excitation of extensors or inhibition of flexors is not consistent with the reflex behavior described by Sherrington (1910). The FRA concept has been criticized in the past because of the terminology (Matthews, 1972 ; Binder, 1982; Lundberg 1979).

However, it is important to appreciate the concepts that a number of different afferent systems can produce common actions and that these common effects to flexor and extensor motoneurons are independently controlled from supraspinal centers (Lundberg,1979; Lundberg,Schomburg 1987).

The type of stimulation that evokes the flexion reflex has been shown to be quite varied; low threshold cutaneous (Hagbarth 1952, Holmquist and Lundberg 1961), high threshold cutaneous ,muscle and joint (Eccles et al 1959) as well as low threshold muscle including group II afferents (Schomburg and Lundberg 1987). A cutaneous nerve trunk can contain afferents from many different receptive fields that converge onto FRA pathways as well as specialized pathways. There is no way of discriminating low threshold FRA afferents from specialized pathways using electrical stimulation of nerve trunks. Because of this we felt it was important to compare the cutaneous effects from a number of different cutaneous nerves on a population of motoneurons that included flexors and extensors. In this way it was possible to evaluate an important concept of the flexion reflex wherby stimulation of large receptive fields produces common actions in similar motoneurons.

In this study a survey of synaptic effects resulting from low threshold electrical stimulation of cutaneous afferents was done. Several cutaneous nerves innervating the hindlimb were stimulated individually with their resultant effects recorded in motoneurons innervating the hamstrings. The latter muscle group is made up two major muscles, one group that flexes the knee, as well as a group that extends the hip. Therefore, by analyzing the effects from many different cutaneous nerves, it was possible to identify common actions in a flexor and an extensor, a concept based on the flexion reflex including alternate FRA pathways. Low threshold stimulation of cutaneous afferents was used as this has been shown clinically to undergo the most change following transection of the intact animal. The preparations used were acute and chronic transected animals as well as intact unlesioned animals so as to assess the effect of long term changes on the pattern of synaptic effects.

In an attempt to predict which neuronal circuits maybe involved in the transmission of cutaneous effects two different muscle afferent pathways were examined in a limited number of flexor and extensor motoneurons of the chronic spinal cat. Firstly, because low threshold cutaneous pathways as well as effects from group II muscle afferents have been shown to be part of the flexion reflex, (Lundberg and Schomburg 1987) the latter was studied from two muscle groups, ankle extensors as well as from knee extensors. Secondly effects from Ib muscle afferents pathways from the ankle extensors were examined as a possible pathway for low threshold cutaneous effects that have a localized effect on motoneurons that is not consistent with the flexion reflex.

Methods

A total of 20 cats weighing between 2.0-3.5 kg were used in this study. Twelve cats underwent L1-L2 spinalization 6-8 weeks prior to the acute experiment. Two cats were spinalized at the L1-L2 level on the day of the acute experiment whereas 6 cats were used as intact preparations.

Surgery for Chronic Spinalization

After fasting for twenty-four hours, the cat was prepared for surgery under aseptic conditions. Sodium pentobarbital (30 mg/kg, intraperitoneal) and a mixture of halothane, nitrous oxide and oxygen was used as an anesthetic. EKG was monitored throughout the surgery on an audio amplifier. The cat was shaved over the spinous processes of T12-L4 and the skin prepared with a betadine solution. A small incision was made over the thoracolumbar processes, with muscle and connective tissue separated using blunt dissection. Once the lamina was exposed it was removed until the surface of the cord was seen. Using fine forceps a complete transection of the spinal cord was made at the L1-L2 level. Often a small amount of xylocaine was applied to the cord topically to avoid any reflex behavior during the transection. The site of the transection was then packed with surgicel to prevent bleeding postoperatively. The wound was then closed in layers using 4-0 silk. The animals were allowed to recover for 6 weeks before the acute intracellular experiment. Nursing care consisted of twice daily manual emptying of the bladder; no infections resulted from the surgery.

Preparation and Surgery for the Acute Experiment

Dissection, data collection and analysis were identical in the transected and intact experimental groups. Two cats were acutely spinalized at the L1-L2 level on the day of the acute experiment. This was done following the lumbar laminectomy approximately two hours before intracellular recording commenced.

After weighing , the animal was anesthetized with a mixture of halothane, nitrous oxide and oxygen. Atropine (.12 mg) was given subcutaneously to decrease bronchial secretions. Once the cat was anesthetized a tracheotomy was performed. The right femoral artery was cannulated and connected to a blood pressure transducer. Intravenous lines were placed in the right forelimb and hindlimb for infusion of medications throughout the experiment. Often a bladder cannula was inserted to monitor urine output. Dexamethasone (4 mg) was given intravenously to reduce spinal cord edema.

Various combinations of the following nerves of the left hindlimb were exposed and cut in preparation for mounting on bipolar silver electrodes : Muscle nerves, Quadriceps including the branch to rectus (Quad), Posterior biceps and semitendinous (PBST), Semimembranous and Anterior biceps(SMAB), anterior biceps (AB), posterior biceps(PB), semitendinous (ST), semimembranous (SM) Hamstrings (defined as a motoneuron pool activated when both SMAB and PBST nerves were mounted on the same electrode.) Medial gastrocnemius(MG), Lateral gastrocnemius and soleus (LG-S), Plantaris (Plant), Flexor digitorium longus and Flexor hallucis longus (FDHL)

Cutaneous Nerves: Saphenous (Saph), Cutaneous caudo-femoralis femoralis (CCF), Sural (the branch that runs from the sciatic medially through the fat pad) , Perforant (Perf), Sur-Perf (where Sural and Perf were mounted together), Superficial Peroneal (SP).

Mixed Musculocutaneous; Common peroneal (C.P.) and the tibial nerve (Tib). Occasionally the tibial nerve was left intact to test cutaneous receptive fields from the central pad of the foot using a buried cuff electrode.
Note: Quad, and Saph were always mounted using buried cuff

electrodes.

A laminectomy was performed from L4 -L6 to expose the lumbar enlargement. Following a blunt dissection of the muscles and connective tissue surrounding the vertebral bodies the lamina were removed in a step-wise fashion from L6 to L4. A separate incision was made at T10 for placement of the rostral clamp that allowed fixation in the spinal frame. The caudal clamp was placed at the sides of the vertebrae at L4. When the laminectomy was finished halothane anesthesia was discontinued and the cat was switched to chloralose anesthesia (initial dose 40mg/kg with subsequent doses added till the cat reached 80-90mg/kg).

The animal was then placed in a Goteborg type spinal frame (Transvertex Co. Ltd, Stockholm) and secured appropriately to prevent movement during intracellular recording. The skin of the left hindlimb and the back was fashioned into a pool, filled with mineral oil and maintained at body temperature (38 degrees). At this time the cat was placed on a respirator and paralyzed with gallamine triethiodide. Subsequently, a bilateral pneumothorax was performed. End tidal carbon dioxide was monitored and maintained at 2-3% level throughout the experiment. Body temperature was also monitored and maintained at 38 degrees.

Intracellular Recording Procedures

The dura was cut over L7-S1 spinal segments and, using fine forceps small "patches" made between blood vessels through the pia to allow microelectrode penetration and localization of the motoneuron pools to be sampled. Intracellular recordings from lumbar motoneurons were obtained using potassium citrate filled glass electrodes with tip diameters in the range of 1.5-2 microns and resistance values of 2-5 megohms. The indifferent electrode was placed in the paraspinal musculature along side the exposed vertebra. A silver ball electrode, was placed at the junction of the L7 dorsal roots with the spinal cord and was used to measure activation threshold for the peripheral nerves (arrival at the cord of the afferent volley produced by the lowest stimulation). The latency of the postsynaptic potentials in motoneurons are reported with respect to arrival of the peripheral nerve volley at the cord dorsum.

Microelectrode recordings, cord dorsum records and current monitor traces were amplified and displayed on two oscilloscopes usually with two different time bases.

Photographs were taken of the recordings and later the filmed records were enlarged and various measurements taken. The photographs were of four separate sweeps superimposed on each other. Averages were sometimes taken online using a Cromenco computer.

Tracking for specific species of motoneurons was guided by the electrical fields produced by peripheral nerve stimulation. Motoneurons were impaled in cord segments L7 or S1 and their species identified by antidromic activation. Constant current stimuli (0.2ms duration) were applied to the peripheral nerves using electrically isolated stimulators. Potentiometers across the stimulator output on each nerve were adjusted throughout the experiment so that one setting on the stimulator dial would deliver the same threshold of stimulation to all nerves. The threshold for nerve stimulation was determined by examining the cord dorsum electrode recordings and defined as the stimulus required to just produce a deflection of the cord dorsum recording. Sometimes it was difficult to see the threshold for activation of the joint nerve and in these instances latency measurements are not reported. Field potentials were taken outside the motoneurons after the intracellular data had been gathered. These field potentials were then subtracted from the intracellular records, either from the film or by using the computer averages.

EPSPs were examined to ensure that they were not reversed IPSPs by injecting a depolarizing constant current or by examining other inputs in the same cell which showed IPSPs in the hyperpolarizing direction.

In two experiments on chronic spinal cats, electrical stimulation of the central foot pad of the cat was done, using a bipolar electrode which just pierced the surface skin of the pad. This area is innervated by the medial plantar nerve with a small contribution from the lateral plantar nerve and contains only cutaneous afferents.(Engberg 1964) We then compared this to stimulation of the mixed musculocutaneous tibial nerve, which had been placed in a buried cuff electrode. In this way we could differentiate the muscle from the cutaneous effects.

Data Analysis

The polysynaptic pathways from cutaneous afferents onto motoneurons in several cases appear to be made up of a mixture of EPSPs and IPSPs. Even when the effect is mainly depolarizing or hyperpolarizing it was difficult to be sure that there was no competing conductances at the motoneuronal membrane. Therefore effects were classified according to their polarity, with excitation being defined as the greater effect in a depolarizing direction, inhibition being defined as the greater effect in an hyperpolarizing direction. Effects were classified as being mixed if there was little overall change in the baseline membrane potential. All of the PSPs were classified by visual inspection of the film that had been placed under an enlarger. There was no attempt made to quantify the amount of an effect.

The muscle effects from group I and group II afferents were classified according to the following criteria. Group I effects; When group I afferents were stimulated electrically, that is at stimulus intensities <1.5 times threshold of the most sensitive nerve fibers (1.5 T) but not greater than 2 times threshold of the most sensitive nerve fibers. (Jack 1978) The second criteria was that the effect resulting from 2T stimulation did not increase in size with subsequent stimulation at 5 T. (Eccles, Eccles and Lundberg 1957) Thirdly, the latency had to be shorter than that which could be produced by group II afferents ie. the latency was below 2.4ms for excitatory effects and <3.3ms for inhibitory effects, the minimum for group II effects in the caudal L6 group II interneuron. (Lundberg 1987)

Group II effects; Group II afferents were considered to evoke an effect when the PSPs occurred at twice threshold an increased in size when threshold was raised between two and five times threshold. (Eccles, Eccles and Lundberg 1957) In instances where group I PSPs were present the group II effect was classified as to what it added onto the group I PSP in that the effect from low threshold stimulation was not included in the classification of the group II PSP. In most cases it was a clear EPSP or IPSP , however there were some mixed effects as well. Latency measurements given for group II data are approximate as in many cases group I effects obscured the earliest latency produced by group II stimulus strength.

Classification of Motoneurons

The hamstring muscles are a large group of muscles on the posterior thigh that have complex functions involving hip extension with knee flexion (PBST)or hip extension with knee extension (SMAB). The various muscles are involved in complex movements for example PBST has a hip extensor function that according to Sherrington 1910 stabilizes the hip to allow stronger knee flexion. Classifying these motoneurons as belonging to merely flexors or extensors underestimates their functional complexity (see discussion). However, to simplify the description of the data we have classified the motoneurons as flexors and extensors from antidromic stimulation of SMAB or PBST nerves that had been dissected free from surrounding tissues and placed on bipolar stimulating electrodes. In cases where PBST and SMAB nerves were placed on the same electrode the motoneurons were identified according to their group I input from quadriceps (stimulus intensity of 2T or less than 2T) after they had been antidromically identified as belonging to the hamstrings motoneuron pool (PBST +SMAB nerves were placed on the same electrode).

Based on this method of classification there were two groups of motoneurons identified, those that received disynaptic inhibition and those that received polysynaptic inhibition from low threshold Quad stimulation. If they received an IPSP at less than or equal to 1.8 ms following low threshold stimulation (2T or less than 2T) from the knee extensor Quad they were classified as motoneurons receiving disynaptic inhibition from quadriceps (Eccles and Lundberg 1958) presumably of group I origin. This group of motoneurons will define a presumed knee flexor (PBST) motoneuron population based on the following: Impulses from low threshold Ia afferents produce inhibition in antagonist muscles ie. knee extensor (Quad) is antagonist to knee flexor PBST (Lloyd 1946 and Eccles and Lundberg 1958). Although we did not specifically stimulate Ia afferents, they would be included in stimulation of muscle afferents at twice threshold. There is a possibility that the IPSPs we classified could be from Ib afferents that would also be included in a stimulation strength of 2T. However, the time course of the Ib IPSPs are more prolonged, than that of the Ia IPSP (Eccles, Eccles and Lundberg 1957). Similarly, disynaptic IPSPs could also be due to renshaw inhibition as the ventral roots were not cut in the experiments. However, IPSPs due to recurrent inhibition have a longer duration (Eccles, Fatt and Koketsu 1954). The shape of the disynaptic IPSPs in the data were characteristically large often greater than 2mv with a short duration (compare fig 1 panel A which is an IPSP at 1.3ms with figure 5 panel A which is an IPSP at 2.5 ms).

If the hamstring motoneurons received inhibition greater than 1.8 ms when Quad was stimulated at group I strength, they were classified as motoneurons receiving tri- or polysynaptic inhibition from quadriceps . These cells will be classified as extensors, due to there lack of disynaptic inhibition from the knee extensor Quad. Note that when Quad was mounted rectus was included. Stimulation of rectus can result in disynaptic inhibition of all the hamstring muscle groups. (Eccles and Lundberg 1958)

However, their is a group of hamstring muscles that do not receive disynaptic inhibition from quadriceps and/or rectus with earlier reports giving no data as with regards to the frequency of this occurring (Eccles et al 1958). (see discussion)

Other extensors were further identified as AB motoneurons if they received group I heteronymous excitation from quadriceps stimulation (Eccles and Lundberg 1958). Although these muscles are considered to be extensors according to Sherrington, they were classified according to their group I effects from Quad stimulation.

It is important to note that the cutaneous nerves tested in this study represent innervation from different skin areas. Tibial, sural and SP innervate the foot, plantar and dorsum surfaces respectively, as well as the lower leg. CCF innervates the posterior thigh and occasionally down to the foot. Saph innervates the front and medial aspect of the thigh. Therefore the latter two nerves generally innervate skin that may overly the hamstring muscle groups. For a more detailed description of skin areas innervated see(Reighard and Jennings 1938 ; Ekholm (1967).

Results

The purpose of this thesis was to survey low threshold cutaneous effects in motoneurons innervating a flexor and extensor muscle and to determine if there were differences in the patterns of effects between transected cats (acute and chronic) as well as spinal cord intact cats.

Stimulation of low threshold cutaneous afferents of the hindlimb produced the following patterns of synaptic effects in presumed flexor and extensor motoneurons innervating the hamstring muscles: 1. predictably produce EPSPs in flexor motoneurons. 2. produce in a nonpredictable pattern IPSPs or mixed effects in extensor motoneurons. 3. produce EPSPs in a small number of extensors in a predictable fashion.

In general there were no differences in these patterns of synaptic effects between preparations following cutaneous stimulation except from two nerves, tibial (Musculo-cutaneous nerve) and CCF (cutaneous). Typically in unlesioned preparations effects from these two nerves could not be elicited at twice threshold stimulation. Following acute and chronic transection of the spinal cord, excitatory pathways to flexors and inhibitory and excitatory pathways to extensors were found.

With regards to muscle afferent pathways it was found that group II muscle afferent pathways examined in the chronic spinal animal were similar to those described in the acute spinal animal (Lundberg 1987). In contrast group Ib pathways from ankle extensors have a different pattern of effects than that previously reported in the acute spinal animal.

Common Post Synaptic Effects from Low Threshold Cutaneous Nerve Stimulation in Motoneurons Innervating Hamstring Muscles

The following data represents effects from muscle and cutaneous inputs in motoneurons innervating the hamstring muscle group. The motoneurons were classified according to either direct antidromic stimulation of PBST or SMAB peripheral nerves or according to their group I input (stimulus intensities of 2T or lower) from quadriceps. This resulted in two groups of motoneurons (total =110) with 61% classified as flexors based on the presence of disynaptic inhibition from Quad (<1.8ms) (Eccles, Eccles and Lundberg, 1957) and 39% as extensors. Of the 27 motoneurons that were antidromically identified as PBST 4 (15%) did not receive group I disynaptic inhibition from Quad and were classified as "presumed extensors". Similarly, of the 25 motoneurons antidromically identified as SMAB 5 (20%) received disynaptic inhibition from group I Quad stimulation and were subsequently classified as "presumed flexors."

Figures 1 and 2 show examples of common effects from several cutaneous nerves in a motoneuron antidromically identified from PBST, in an acute spinal cat (fig 1). This motoneuron receives disynaptic inhibition from quadriceps stimulation at twice threshold (panel A, 1.3ms latency) as well as excitation from quadriceps muscle afferents when the stimulus strength is raised to 5 times threshold to include group II muscle afferents (panel B) (see methods). Stimulation of the tibial nerve produced EPSPs at a latency of 3ms which increased in amplitude and caused the cell to fire when the stimulus was raised to 5T(panel D). Similar results were seen from stimulation of Saph (panel E, F) and Sur-Perf (panel G, H).

Figure 2 shows records from a motoneuron antidromically identified as innervating the hamstring muscle group, in a chronic spinal animal transected at the L1-L2 level 6 weeks prior to the experiment. Stimulation of Quadriceps at group I strength (2T, panel A) produces disynaptic inhibition at 1.3ms latency. Tib nerve stimulation (panel E) at 2T produced substantial excitation at a latency of 2.3 ms. Other cutaneous effects are also excitatory at 2T,(panel I, K, M, O), increasing their amplitude at 5T (panel H, J, L, N, P).

In comparing the effects found in these two motoneurons in figures 1 and 2, from acute and chronic transected cats, the gross similarity between PSPS is evident. It should be noted that the cutaneous nerves tested in these two motoneurons innervate the entire hindlimb. The similarity of these EPSPs resulting from stimulation of such a large cutaneous receptive field is consistent with convergence onto common interneurons involved in the flexion reflex (Eccles and Lundberg, 1959). This pattern of excitation from a wide receptive field (at least two different cutaneous inputs) was seen in 40 out of 43 motoneurons classified as flexors (see methods) in transected animals. Of this group of motoneurons, 22 were antidromically identified as innervating PBST with 19 motoneurons receiving a common pattern of excitation from at least two different cutaneous inputs.

Compared to other cutaneous nerves, Saph stimulation produced the highest incidence of inhibition or mixed effects, in 6 out of 24 motoneurons classified as presumed flexors in the chronic spinal animal. The example in figure 2 (panel N) shows a predominantly excitatory response, with a steep fall from the peak of the EPSP. This is likely due to a strong inhibitory component. EPSPs produced by stimulation of other cutaneous nerves do not show this inhibition at similar stimulus strengths (compare panel N with panel J). Note that the effect in panel O is not an IPSP. SP stimulation at 2T strength produced an action potential (spike not shown in the figure) followed by an afterhyperpolarization that is not an IPSP. Inhibition of flexor motoneurons in decerebrate, acute spinal cats, has been described in terms of a localized effect (Hagbarth, 1952; Ekholm, 1964) which predicts inhibition of PBST motoneurons when skin overlying the antagonist extensor muscle, which is innervated by Saph (Ekholm 1964), is stimulated.

Eccles and Lundberg (1959) originally described the effects from FRA afferents as being predominant excitation of flexor and inhibition of extensor motoneurons. These authors also noted that extensors may also get excitation from FRA afferents (Holmquist and Lundberg, 1961). In the present data we have 6 examples of excitation from a large cutaneous receptive field onto motoneurons innervating SMAB and as such classified as extensors by Sherrington (1910). However, in the present study they were classified as presumed flexors (see Table 1), because these 6 motoneurons received disynaptic inhibition from quadriceps. One of these is illustrated in figure three from an Sm motoneuron in a chronic spinal cat that receives disynaptic inhibition from Quad (panel K). Cutaneous stimulation produces excitation from Tib (panel A-C), Saph (panel D), Perf (panel E), Sural (panel F), Joint (panel I), and SP (panel L). Another example of excitation in an "extensor" according to Sherrington (1910) from a variety of ipsilateral cutaneous nerves is illustrated in figure 4 from a chronic spinal animal. This cell can be classified as an AB motoneuron on the basis of a monosynaptic EPSP from Quad (panel E), (Eccles, Eccles and Lundberg 1957) but also receives longer latency inhibition from Quad (panel E). The effects found in this motoneuron demonstrate excitation from Tib at low threshold (panel A, latency 2.0 ms), SP (panel C, 2.3 ms), CCF (panel F, 2.4ms), Joint (Panel G), Sur-Perf (panel H, 2.0 ms) as well as Saph (panel D, 2.0 ms); the latter showing a late inhibitory component.

Table I gives the means of the earliest latencies of cutaneous PSPS elicited at 2T stimulation and their frequency of occurrence in flexor motoneurons of transected and unlesioned cats. The latencies for EPSPs, the predominant effect in presumed flexors, when the cutaneous input is pooled was from 2ms -3ms. The 20% of cells that were identified as SMAB antidromically as well as receiving disynaptic inhibition from Quad, were all found in the acute spinal cat except for the one shown in figure three. In contrast to the flexors that received predominantly EPSPs from cutaneous inputs, the SMAB motoneurons that received disynaptic inhibition from Quad received either inhibitory or mixed effects from other cutaneous inputs (see Table I acute IPSPs and/or mixed effects). AB motoneurons(6out of 11), identified by the presence of a monosynaptic EPSP from Quad stimulation received disynaptic inhibition from Quad (Eccles ,Eccles and Lundberg 1957) as well as EPSPs from the other cutaneous inputs.One AB shown in Table 1 although it received disynaptic inhibition from Quad also received IPSPs from all cutaneous nerves tested. The 15% of antidromically identified PBST motoneurons, that did not receive disynaptic inhibition from Quad were all found in chronic spinal animals. They all received excitation from other cutaneous inputs except for 1 motoneuron that received predominantly inhibition from different cutaneous afferents.

In summary, low threshold (2T) cutaneous stimulation produces common excitatory effects in motoneurons that receive disynaptic inhibition from Quad. This occurs mostly in flexors identified antidromically as well as according to group I disynaptic inhibition from Quad. One group of "presumed flexors" antidromically identified as innervating SMAB motoneurons in acute spinal cats, received inhibitory or mixed effects from the majority of cutaneous inputs. In contrast, another group of "presumed flexors" identified as AB motoneurons in chronic spinal animals, received disynaptic inhibition from Quad as well as excitation from the majority of cutaneous inputs. These observations point out the difficulty in predicting cutaneous reflex effects based on the classification of motoneurons as flexor or extensor (Sherrington 1910) more so for extensors, than flexors, especially in acute spinal cats (see discussion, Classification of Motoneurons).

B. Variable Pattern of Postsynaptic Potentials Produced by Low Threshold Stimulation of Cutaneous Nerves in Presumed Extensor Motoneurons

Motoneurons that did not receive disynaptic inhibition from Quad (see methods) were classified as presumed extensors. This included five AB motoneurons identified on the basis of monosynaptic excitation from Quad. (Eccles et al 1957) This group of motoneurons received a mixture of EPSPs and IPSPs from cutaneous stimulation in acute and chronic transected animals. There were four antidromically identified PBST motoneurons that were classified as "presumed extensors" . They all received excitation from all cutaneous inputs except for one motoneuron, and therfore were flexor-like in terms of the flexion reflex. Another finding in this group of motoneurons was that of all the cutaneous nerves tested, twice threshold stimulation of the tibial nerve was most consistent in producing inhibition of presumed extensor motoneurons in acute and chronically transected cats (25 out of 33 motoneurons of presumed extensors Table II).

Figure five is an example from a chronic spinal animal of a variable pattern of PSPS in a hamstrings motoneuron that does not receive disynaptic inhibition from Quad. Stimulation of Quad (panel A) at twice threshold produces a small monosynaptic EPSP as well as inhibition at a latency of 2.5 ms. The middle trace in panel B shows the absence of an extracellular field potential from stimulation of Quad. This motoneuron was classified as innervating AB. One group of nerves, joint, SP, and Tib (panels C, G, K) produced predominant inhibition with stimulation at twice threshold. The latency for the Tib IPSP was 2.5ms (panel K). The size of the IPSP increased as the stimulation strength was increased to 5T (panel L), with the small initial positive deflection being a field effect (not illustrated). This type of IPSP at 2T was found in 70% of motoneurons classified as extensors. Sp stimulation also produced inhibition at 2T (panel G) with a latency of 5 ms. Of 17 motoneurons classified as extensors, thi type of IPSP from SP stimulation occurred in 47% of motoneurons, predominant EPSPs were seen 29% times, 17% were mixed effects and 1 no effect (see Table II). The effect seen at 5T following stimulation of the SP nerve (panel H) also shows an increase in the size of the IPSP that is similar to Tib, but the initial EPSP here is not a field effect. Although SP stimulation produces IPSPs in extensors it also has a high incidence of EPSPS and mixed effects as compared to tibial stimulation at 2T (see Table II).

In figure 5 another group of cutaneous nerves gave mixed or excitatory effects. Saph gave mixed effects at 2T stimulation, (panel F) made up of a small inhibitory component followed by an EPSP. CCF and Sur-Perf produced EPSPs although the former gave what looked like a mixed effect in one trace, and Sur-perf shows a sharp fall from the peak of the EPSP which also suggests an inhibitory component. In transected cats stimulation of Saph, and Sur-Perf at twice threshold produced an equal distribution of EPSPs, IPSPs and mixed effects in motoneurons classified as extensors (see Table II). In contrast CCF stimulation gave excitation in all 8 motoneurons tested.

The data in Table II emphasizes the variable effects seen in extensor motoneurons following cutaneous stimulation indicating multiple pathways, that are not seen with such a high incidence in the flexor motoneuron pool (compare TABLE I-II) . The distribution of latencies in presumed extensor motoneurons of transected animals was, in contrast to the flexors of transected animals, quite variable. The mean latencies of IPSPs from SP (4.29 ms), Saph (3.96 ms) and Sur-Perf(4.50 ms) were longer than the effects from tibial stimulation (latency 2.82ms acute, 2.84 ms chronic).

The pattern of PSPS seen in extensors from low threshold cutaneous stimulation can be quite variable and cannot be predicted simply by the FRA reflex pattern of inhibition of extensors. Figures 3 and 4 demonstrate an excitatory convergence from all the cutaneous nerves tested in extensors that receive disynaptic inhibition from Quad. Figure 5 demonstrates predominant inhibition from SP, Tib, and joint as well as predominant depolarization from CCF, Sur-Perf and Saph. In no case were we able to predict a consistent pattern of PSP effects from cutaneous nerve stimulation, in the same extensor motoneuron. However, tibial stimulation produced low threshold inhibition more often and at a shorter latency, in extensor motoneurons than any other cutaneous nerve tested.

C. Comparison of Unlesioned and Lesioned Cats

Qualitatively, in cats without spinal lesions, the pattern of PSPS is similar to that described in the spinal transected cats; excitation in presumed flexors and variable effects in presumed extensors motoneurons when low threshold cutaneous afferents are stimulated. Following transection there are some striking differences between unlesioned and lesioned animals, depending on the cutaneous nerve tested. For example, effects from Tib and CCF were rare in the unlesioned preparation, and frequent following transection in both flexor and extensor populations (see below). Saph, Sur-Perf and Sp effects on the other hand appeared more frequently in the unlesioned cat especially with regards to the flexor population.

All the motoneurons in the intact preparation were identified according to synaptic effects from group I quadriceps afferents. All motoneurons receiving disynaptic inhibition from Quad received predominant EPSPs from SP, Sur-Perf and Saph stimulation (refer to Table I). In extensor motoneurons, ie. those that received IPSPs with a latency > 1.8ms from group I Quad stimulation, variable effects were seen from stimulation of the same nerves. Stimulation of Sur-Perf, SP and Saph are similar in the transected cats and the unlesioned animals with regards to the incidence of EPSPs in flexors.

In spinal cord intact preparations low threshold stimulation of Tib and CCF, resulted in either no effect, or inhibition of both extensor and flexor motoneurons. In the unlesioned animal CCF stimulation produced EPSPs in 1 out of 8 flexors and 1 out of 6 extensor motoneurons. In contrast, the incidence of CCF excitation was much more frequent in the chronic spinal preparation with EPSPs in 11 out of 16 flexors and 8 out of 8 extensor motoneurons. Thus low threshold CCF effects are less frequent in the unlesioned cat in both flexor and extensor motoneuron pools. Another example of a low threshold cutaneous effect that is more frequent following transection is seen following Tib stimulation. In a total of 22 presumed flexor motoneurons (Table I) from unlesioned preparations 2T tibial stimulation gave excitation only 2 times, inhibitory or mixed effects 4 times and was without effect 8 times. In 9 of these cells raising the stimulus strength to 5T produced excitation in 4 motoneurons, 3 of which were classified as flexors.(see figure 6) However, in acute and chronic transected cats twice threshold stimulation caused EPSPs in 38/48 motoneurons classified as flexors and in 2/33 classified as extensors. In comparing the results of tibial stimulation between acute and chronic transected cats, there was no difference in the threshold to produce the EPSPs or the earliest latency for the effect. This implies that the excitatory pathway is released immediately from a descending control following transection, especially the pathway to flexors, or motoneurons receiving disynaptic inhibition from quadriceps.

Table I gives the means of the earliest latencies of PSPS elicited at 2T stimulation and there incidence of occurrence, in presumed flexor motoneurons of transected and unlesioned cats. This table illustrates that changes in latencies of EPSPs, the predominant effect found in flexors, between preparations depends on the cutaneous nerve tested. For example, Saph has a similar mean latency for EPSPs between chronic and unlesioned preparations whereas there is a longer latency in the acute spinal preparation (p>.005, t-test). Sur-Perf is similar to Saph. In contrast, tibial stimulation produced similar mean latencies for EPSPS in both acute and chronic spinal animals (2.62 and 2.72 ms respectively).

In the presumed extensors of Table II it can be seen that the differences in earliest latencies between all three preparations were variable depending on the nerve tested. Stimulation of the tibial nerve that produced IPSPs in the presumed extensors showed no difference between preparations. There appears to be a statistically significant difference between latencies of IPSPs in the chronic spinal and unlesioned groups produced by stimulation of Saph, albeit the number of effects tested is small for each group. Sur-Perf demonstrates more EPSPs following chronic transection with a shorter latency although their was only one effect found in the unlesioned preparation. In contrast CCF stimulation produces a higher incidence of EPSPs but at a longer latency than in the unlesioned.

Analysis of the effects of spinal transection on cutaneous pathways to extensor motoneurons is more difficult than that of flexors because of the greater variety of synaptic effects seen in extensors. In order to get a general picture of the incidence of effects, the observations from different nerves were pooled together in the unlesioned and chronic spinal preparation(TABLE II) In the unlesioned animal EPSPs were seen 17% of the time, IPSPs 48%, mixed effects 21% no effect 14% (29 observations). Following transection, 45% were IPSPs 30% were EPSPs 20% were mixed effects and no effect 5% (82 observations). This slight increase in the incidence of EPSPs occurs mainly following stimulation of CCF but also Saph and Sur-perf but not from tibial and SP stimulation.

In summary, Tib and CCF stimulation appear to be affected by transection with an increase in incidence of EPSPs to flexors (eg. Tib) and an increase in excitation to flexors and extensors (eg. CCF). In the flexor population Saph, Sur-Perf and SP appear more frequently in the unlesioned preparation than Tib or CCF. Cutaneous effects in extensors are quite variable in both lesioned and unlesioned preparations. Therfore it is difficult to make any conclusions as to the effects of transection. In general there is an increase in EPSPs following stimulation of Saph, Sur-perf and CCF, in the presumed extensor population following transection. Tibal and Sp stimulation result in if anything a greater incidence of IPSPs.

D. Electrical Stimulation of Skin Afferents

The tibial nerve is a mixed muscle and cutaneous nerve innervating the short extensor muscles of the foot, skin on the plantar surface of the foot, as well as the central pad and toe pads of the sole of the foot which are richly innervated (Boyd and Davey 1968) There are three times as many myelinated extramuscular and /or cutaneous afferents as there are motor afferents in the tibial nerve as we mounted it.(Boyd and Davey 1968) In order to see if cutaneous afferents can produce the low threshold excitation seen in the flexor motoneurons innervating hamstrings, the tibial nerve was dissected free from surrounding connective tissue but left intact to its distal innervations and mounted in a cuff electrode. This allowed a comparison of the effects resulting from stimulation of the skin versus the entire nerve (motor component included). Fig. 7 panel A show excitation at a 2.1ms latency in an PBST motoneurons from a chronic spinal cat following electrical stimulation of the central pad of the foot. Stimulation of the whole tibial nerve via the cuff electrode also produced excitation at the same latency (panel B). This figure demonstrates the cutaneous origin of the EPSPs produced from tibial stimulation and that this effect can occur from a small circumscribed area of skin.

E. Effects from Muscle Afferents in Transected Cats

Because cutaneous afferents are known to make connections with interneurons interposed in muscle afferent reflex pathways, such as the Ib and Ia inhibitory interneurons, (Baldissera et al 1981) we examined the incidence of group I and group II effects following muscle afferent stimulation in chronic transected cats. Table III summarizes the effects from muscle afferent stimulation of the triceps surae on presumed flexor and extensor motoneurons in chronic spinal animals. Excitation following stimulation of group II muscle afferents is significantly more prevalent in flexors, whereas group II effects in extensor motoneurons are inhibitory and/or mixed PSPS. It is interesting to note that this is parallel to the findings of the cutaneous effects, that is excitation in flexors and variable effects in extensors. In contrast group I excitation and inhibition from triceps surae afferents appear to be depressed in the chronic spinal animal especially with regards to flexors.

The AB motoneuron in figure 5 from a chronic spinal animal, illustrates an example of Ib inhibition from MG. When MG was stimulated at lower stimulus strengths from 1.2T to 2T, the IPSP increased in size. (panel M-P) Increasing the strength of stimulation to 5T only produced a small change in amplitude. Therfore, lower thresholds that activate mainly group pI afferents produced the major synaptic effect. Further, the latency for the effect was 1.3 ms which is below the minimum latency recorded for group II inhibitory effects (Lundberg and Schomburg 1987).

Table III illustrates a striking lack of Group I excitation from triceps surae onto motoneurons that receive disynaptic inhibition from quad. Eccles et al first reported excitation from group I triceps surae afferents in PBST motoneurons in spinal transected cats anesthetized with phenobarbitol in 14 out of 58 motoneurons(24%). (Eccles 1959) Jankowska and McCrea, using low spinal chloralose anesthetized cats as well as 4-aminopyridine reported a higher incidence of excitation from triceps surae onto PBST motoneurons in 31 out of 34

PBST motoneurons (91%). In the data represented in Table III only 4 EPSPs were found from stimulation of group I afferents from MG or LGS.(13%) Therfore, the pathway has been shown to exist in the acute spinal cat under different experimental conditions, but in the chronic spinal cat when the effects of spinal shock have been reduced, the incidence of group I excitation is decreased as compared to the acutely transected cat.

The motoneuron illustrated in figure 2 shows an example of group II excitation from stimulation of quadriceps (panel B) as well as probably FDHL (panel C), at an approximate latency of 3-4 ms. Since we did not dissect the interosseous nerve from FDHL it is difficult to know whether the late excitatory effect is from extramuscle receptors or group II spindle receptors (Harrison and Johannisson 1983) However, this late effect is not from group I afferents. (Note the clear separation between the early latency group I excitation and the longer latency excitation which occurs at 5 times threshold.) In this cell but not shown in the figure, stimulation of MG and LGS at group I strength (2T) produced no effects. Stimulation at group II strength of the LGS nerve produced a small EPSP whereas Mg stimulation again produced no effect. As can be seen from Table III, muscle afferent input to flexor motoneurons appears to be the strongest from quadriceps group II afferents in 10 out of 12 motoneurons where the effects were tested.

An example of a group II mixed effect from Quad stimulation is shown in fig 5 panel B, a pattern that was seen in four extensor motoneurons. Note the widening of the IPSP at 5-6 ms from slower conducting afferents with the addition of a late EPSP. However, in four other extensor motoneurons stimulation of Quad at 5T produced an IPSP at 3 ms without the later EPSP.

In all there were 10 motoneurons which received group II excitation from Quad muscle afferents and disynaptic inhibition from Quad group I muscle afferents that also received excitation from cutaneous afferents. In those motoneurons where there were mixed or inhibitory effects from Quad group II muscle afferents there were also mixed effects from cutaneous inputs, as well as the longer latency inhibition from group I Quad stimulation. These pattern of effects is what you would predict based on an underlying concept of the FRA hypothesis, that stimulation of cutaneous and muscle afferents may produce in the same species of motoneurons similar effects. (Holmquist and Lundberg 1961: Lundberg 1975)

Discussion

Sherrington's earlier studies on cutaneous reflexes described the flexion reflex, whereby, excitation of flexors and inhibition of extensors resulting from stimulation of muscle and cutaneous afferents, as a useful concept in understanding the organization of segmental reflex pathways. Intracellular studies extended these earlier observations to show that there were afferent inputs that produced common actions opposite to the flexion reflex, that is excitation in extensors and inhibition in flexors (Holmquist and Lundberg 1961). These pathways were included in the description of the flexion reflex and termed "alternate FRA" pathways, emphasizing the common action from different afferents (Lundberg 1979). However, there was also evidence that there were other cutaneous reflexes more localized in their effects, which resulted from low threshold stimulation (Engberg, 1964 ; Hagbarth 1952). When using electrical stimulation of cutaneous afferents, low thresholds do not identify a particular group of receptors (Boyd and Davey 1968) nor is there any difference in the effect on a monosynaptic reflex condition test paradigm between low and high threshold(Hagbarth 1952). The best predictor of a cutaneous effect on the monosynaptic reflex was the location of the receptive field

(Hagbarth 1952).

In this study I have stressed the importance of a group of afferents producing a common effect (Lundberg 1987) to include alternate FRA pathways as well as the typical FRA pathways.To differentiate the common actions of the FRA from the localized actions using low threshold stimulation, the effects from many cutaneous nerves were analyzed in a flexor and an extensor muscle. Upon stimulation of low threshold cutaneous afferents that innervate the hindlimb of the cat we have found the following pattern of effects in flexor and extensor motoneurons innervating the hamstrings: the afferents predictably produce EPSPS in flexor motoneurons, afferents produce in a non-predictable pattern IPSPs or mixed effects in extensor motoneurons, or the afferents produce EPSPs in a small number of extensor motoneurons of the hamstrings. This same pattern of PSPs in flexors and extensors of the hamstrings as well as in triceps surae has been shown by other investigators(Schomburg and Steffens 1986), using low threshold natural stimuli in acutely transected high spinal cats.

Based on what is known about cutaneous reflexes in animals with transection of the spinal cord, several hypotheses were discussed in the introduction to this thesis. Flexion of the cat's hindlimb in a transected animal occurs with gentle manipulations of the cat's toes. Presumably the same flexion response in a intact animal is seen possibly as a withdrawal mechanism from higher threshold noxious stimuli. Therfore, it was hypothesized that there would be differences in the pattern of synaptic effects following low threshold cutaneous stimulation between lesioned and intact animals. Because there were differences between flexion and extensor reflexes, the latter emerging later than the former, and that cutaneous reflexes may take up to a week to appear following transection, it was also hypothesized that there would be differences between acute (immediately following transection) and chronically transected cats (six weeks post transection) following stimulation of cutaneous afferents. However, overall there were no differences in latencies or occurrence of synaptic effects between the three different preparations, but there were exceptions in both flexor and extensor muscle groups. One such exception occurred following stimulation of the tibial nerve in the flexor group, which appeared to be inhibited in the intact preparation, but which produced EPSPs in almost all motoneurons tested in the transected animals. Differences between preparations in the extensor group were difficult to analyze due to the variability of reflex effects, but there were some significant changes with regards to stimulation of tibial and CCF nerves. The following discussion will first describe the pattern of synaptic effects found, resulting from low threshold cutaneous stimulation, in relation to localized pathways and FRA pathways, to be followed by a comparison of these effects between the different preparations.

Low Threshold Stimulation of Cutaneous Afferents Produces an FRA-Like Effect in Flexors

The data shows that flexors receive a powerful excitation from stimulation of low threshold cutaneous afferents that is consistent with what the high threshold effects from the FRA would predict. From Table 1 it can be seen that SP, Saph and Sur-Perf produced a high incidence of EPSPs in presumed flexors in transected cats as well as in unlesioned cats. In contrast tibial and CCF stimulation were inhibited in the unlesioned animals, but also produced a high incidence of EPSPs in transected cats. (see below)

As can be seen from figure 1, both 2T and 5T stimulation predictably produces excitation from stimulation of cutaneous afferents in flexor motoneurons. This is evidence for the idea that low threshold cutaneous afferents should be included with the higher threshold (ie. 5T) afferents of the flexion reflex with regards to their effect on flexors (Schomburg 1986, Hongo et al 1969). What other evidence is there that would classify low threshold afferents as belonging to FRA ?

Engberg (1964) studied the effects from stimulation of the central foot pad, which is innervated by a branch of the tibial nerve, onto muscles of the foot and hindlimb of the cat. He found using low threshold stimulation of the central pad of the foot that no PSP effects could be found in other muscles of the hindlimb including PBST (see Engberg 1964, fig 11 panel F). To produce excitation in PBST he increased the stimulus strength, which produced an EPSP at a latency of 6ms. The preparation he used was an acute spinal cat that had been transected following decerebration, therfore not requiring an anesthetic. Our data clearly contradicts this finding in both the acute and chronic spinal cord transected cat with one difference being that we used chlorolose anesthesia. Using twice threshold stimulation but also lower stimulus strengths (see fig 7), we have found EPSPS in PBST motoneurons following electrical stimulation of the foot pad as well as whole nerve stimulation. The latency for the effect was also shorter (2.1ms fig 7) than Engberg's (1964) higher threshold effects. Engberg's(1964) results led him to conclude that low threshold stimulation of the PAD was NOT an FRA effect because they did not excite flexors such as PBST. Our data however, suggests that low threshold PAD stimulation does produce "FRA-like effects" at least in PBST motoneurons in both acute and chronic transected animals. Because we have not examined other flexor groups we have no evidence that this pathway evokes the en mass flexion reflex behavior, or if it is in fact active during the flexion reflex. Therfore, future studies are required to describe this pathway as being a part of the flexion reflex but synaptic effects presented in this paper indicate that it is at least a parallel pathway to the high threshold FRA effects described in PBST motoneurons (Eccles and Lundberg 1959).

Inhibition from low threshold cutaneous stimulation occurred rarely, however, stimulation of the saphenous nerve resulted in the greatest incidence of inhibition in the flexor motoneuron pool. This finding is interesting in view of Hagbarth's (1952) description of localized effects to the semitendonosis muscle of the hamstrings (hip extensor and knee flexor). He demonstrated that the skin on the ventral surface of the thigh which overlaps the extensor muscle Quad and is innervated by Saph (Ekholm 1964), results in inhibition of the monosynaptic reflexes elicited from semitendonosis stimulation. Other skin areas from the hindlimb including other skin areas innervated by Saph, consistently facilitated the MSR from ST. It is possible that the Saph nerve contains afferents that produce excitation in ST, as well as afferents that produce the inhibitory localized effects described by Hagbarth (1952).

Low threshold Stimulation of Cutaneous Afferents produces a Common Pattern of Excitation in Extensors

There is evidence in the literature that there are common effects from afferents that will produce excitation in extensors of transected animals. (Holmquist and Lundberg 1961; Wilson, 1963 ; Hagbarth, 1952) These have been documented in a case by case fashion as there was no specific lesion which allowed the effects to be studied. This is in contrast to the flexors where a low pontine lesion releases a common inhibitory pathway to flexors and a lower spinal lesion releases a common excitatory pathway to flexors. Schomburg and Steffens (1986) reported that over two thirds of the SMAB motoneurones in their sample of high spinal cats received excitation from low threshold mechanical stimulation or nocioceptive stimulation. Our data indicates a lower incidence of common excitation in extensors with this pattern occurring in 7 (6 AB and 1 SM) out of 16 extensor motoneurons(11AB cells and 5 SM). However, we may have underestimated the occurrence of excitation from a large number of cutaneous afferents due to the method of classification of motoneurons (see below).

From table II the nerves which produced the largest incidence of EPSPs in extensors of transected cats were CCF, Saph, and Sur-Perf. In each case, there was a low incidence of excitation in the unlesioned preparations which increased following chronic transection. CCF stimulation also produced only EPSPS in transected cats whereas the other nerves had a number of other effects that were classified as IPSPs and Mixed effects. CCF has been shown to innervate the posterior thigh and lower leg of the cat's hindlimb extending as far as the sural nerve, and in some cases including the receptive field of the sural nerve when the latter was thin and extended only a short distance (Ekholm 1967). It is possible that CCF would innervate skin overlying the extensor SMAB and could be responsible for a localized effect to this muscle. Hagbarth (1952) did not test SMAB so their is no evidence that there is a localized effect for this muscle, although there were other examples of extensors with localized effects including gastrocnemius-soleus, and quadriceps.

This example points out the difficulty in defining FRA inputs on the basis of a common effect at the motoneuron. On the one hand we have evidence for a common input from 3 nerves (CCF, Saph, and Sur-Perf) which produces excitation in extensors and could therfore be thought of as an alternative FRA pathway. However, because CCF also innervated skin overlying the extensor SMAB, we cannot exclude the possibility that this is a more localized effect that is seen in transected cats and is in fact a parallel pathway to an alternate FRA pathway.

Low Threshold Stimulation of Cutaneous Afferents Produces a Pattern of Mixed or Inhibitory Effects in Extensors

This type of pattern is contradictory in that there are a group of motoneurons in which we cannot predict the effect of cutaneous simulation in extensors and therfore is not a "pattern". However, they are grouped together for the purpose of discussion with the common denominator being unpredictability. In fact these effects probably represent many different cutaneous pathways and demonstrate the point illustrated by Hagbarth (1952) as to the pitfalls of using electrical stimulation. Varying strengths of electrical stimulation discriminates between large diameter therfore low threshold and first activated afferents from higher threshold small diameter afferents. In the cutaneous nerves there is a considerable overlap in diameters of the afferents so that electrical stimulation does not isolate one set of afferents that innervate a homogenous population of receptors (Boyd and Davey 1968). Natural stimulation is the only reliable method that can isolate a particular set of afferents. However, an exception to this appears to involve the effects from tibial stimulation in extensors, as this nerve produced IPSPs consistently in extensors of transected animals. Possibly this nerve contains a large number of large diameter afferents that allows us to see a "pure effect".

Tibial stimulation produces inhibition in 75% of extensor motoneurons of transected cats at a latency that was much shorter than the other cutaneous nerves (2.82 ms). It is interesting to note that this latency is similar to the latency of EPSPs found in flexor motoneurons following tibial stimulation (2.72 ms). Possibly these pathways maybe linked at the interneuronal level wherby stimulation of tibial afferents will result in excitation of PBST and inhibition of SMAB motoneurons.

Stimulation of Sp also resulted in IPSPs in a significant number (45%) of extensor motoneurons. Similar to the results from Tib stimulation, SP stimulation showed an increased incidence of IPSPs in extensors of transected animals compared to CCF, Saph and Sur-perf which show an increase in incidence of EPSPs following transection. (see comparison between lesioned and unlesioned preparations) However, one important difference between the effects seen from SP and Tib is that the former occurred with a longer mean latency (4.29 ms) as compared to Tib (2.82 ms)

Stimulation of Tib, SP, CCF, and Saph, also produced a number of mixed effects in extensors. (Table II) Each nerve seems to contain receptive fields that produce different synaptic effects so that when stimulated electrically we record an EPSP followed by an IPSP. It often was the pattern that the first effect was an EPSP, but we also have examples of an IPSP as the first effect. (see fig 5 panel F). It is interesting to note that this effect is in a motoneurons classified as an extensor. Based on the typical FRA pattern we would predict an IPSP. The EPSP could be part of other alternate FRA pathways or more localized cutaneous pathways.

Group II Muscle Afferents Produce Common Actions in Flexor and Extensor Muscles of Transected Cats

In the acute transected animal there is evidence to demonstrate that excitatory and inhibitory effects from stimulation of group II muscle afferents are released from descending control.(Eccles and Lundberg 1959. Holmquist and Lundberg 1961) These same studies also demonstrated that cutaneous stimulation produced similar results as the group II muscle afferents. Those motoneurons, only innervating flexor muscles, that received group II excitation from muscle afferents, also received excitation from cutaneous stimulation. Inhibitory pathways from group II afferents have also been recently examined to show that if group II stimulation produced inhibition or mixed effects in motoneurons mostly innervating extensors, they also received inhibition or mixed effects from cutaneous stimulation. (Lundberg and Schomburg 1987, Schomburg and Steffens 1986)

In the chronic preparation used in these experiments, a similar pattern of effects from group II muscle afferents and cutaneous stimulation was found in motoneurons that innervate the hamstring muscles. (Table III, Fig 1 and 2). Motoneurons classified as flexors, received a common excitatory input from group II quadriceps afferents as well as from a number of different cutaneous nerves. Motoneurons classified as extensors received either mixed or inhibitory effects from group II quadriceps stimulation. (fig 5) Although the number is small to differentiate between mixed and inhibitory effects, these same motoneurons received either mixed or inhibitory effects from cutaneous stimulation. A similar pattern of muscle and cutaneous synaptic effects have been reported by others in acute spinal animals. (Schomburg, Lundberg 1987).

Possible Neuronal Circuits that may Mediate Low Threshold Cutaneous Effects

As discussed previously in the introduction, muscle afferent pathways have been shown to receive convergence from low threshold cutaneous afferents. Interneurons interposed in muscle afferents pathways have been shown to receive convergence from subpopulations of flexor and extensor muscle afferents as well as low threshold cutaneous afferents (Lundberg and Schomburg 1987; Edgley and Jankowska (1987) Czarkowska at al 1981: Harrison and Jankowska 1983). Information about convergence at the interneuronal level as well as at the motoneuronal level can be used to hypothesize possible neuronal circuits that could explain the pattern of effects presented in this thesis. The first to be discussed are possible group II pathways.

Group II

Group II interneurons described by Schomburg and Lundberg (1987) in the more caudal L6-S1 segments received their most effective input from group II pretibial flexors, triceps surae and flexor digitorum longus. Recently, Edgley and Jankowska(1987) have found a powerful projection from group II afferents to a population of interneurons in the mid lumbar segments (L3, L4, L5) which in turn project to the PBST and Gastrocnemius motor nuclei in the more caudal lumbar segments (Edgley and Jankowska in press). These midlumbar interneurons characteristically receive strong group II input from quadriceps, sartorius and pretibial flexors as well as from posterior tibial and popliteus (Edgley and Jankowska in press). Based on the powerful group II projection from quadriceps stimulation to flexor motoneurons of the chronic spinal cats, and the similarly strong Quad projection to the L4 interneuron (Edgley and Jankowska 1987), this midlumbar interneuron maybe a candidate for transmission of low threshold cutaneous effects to PBST motoneurons.

Other evidence that the L4 interneuron may mediate low threshold cutaneous effects can be shown on examination of the latencies of these effects presented in tables I and II. The actions of the L4 interneurons onto motoneurons would be delayed because of the intraspinal distance between L4 and L7. From the presented data the tibial nerve provides the best example of a low threshold pathway to flexors and extensors of the hamstring muscle group. In the present study the mean latency for tibial excitation in flexors is 2.72 ms in 31 cells with a range of 2.0 to 3.0 ms. The question is whether this would be too fast to be part of the L4 interneuron. Edgley and Jankowska(in press) recorded from L4 interneurons following antidromic activation from L7 motor nuclei. Based on that data one can estimate the earliest latency that a cutaneous input could arrive at the motoneuron if the L4 interneurons were interposed. This estimate is based on the following considerations as outlined by Edgley et al (in press) . Firstly, the mean antidromic latency was 1ms and from this value the latent period for spike activation (.3ms) should be subtracted producing a calculated intraspinal conduction time of .8ms. Secondly, the earliest latency at which cutaneous input arrives at the L4 interneuron was 1.2ms (measured from a cord dorsum located at L4 dorsal roots). To this one should add .2ms for the delay between the onset of the EPSP and the action potential generated. Therefore 1.4 ms would be the time for activation of L4 interneuron from cutaneous afferent input. Thirdly, an additional .3ms should be added for the synaptic delay between the terminals of the L4 interneuron and the motoneuron. Fourthly, .2ms should be added to the calculated latency to allow for differences in conduction time as the cord dorsum in this study was located at the L7 spinal cord segment. According to these assumptions the minimum pathway is disynaptic with a latency of 2.7 ms (.8+1.4+.2 +.3 =2.7). This fits nicely with our mean latency of 2.72 ms found in flexor motoneurons following tibial stimulation. The range of latencies which we also report, some as low as 2.1ms (see fig 7) could be due to differences in the intraspinal conduction time of the L4 interneuron.

Traditionally, a latency of >1.8ms would be considered to be trisynaptic (Lundberg 1975). However, the L4 interneuron located more rostrally involves a greater intraspinal conduction time which would increase the latency of the effect but would require only two synapses to reach the motoneuron. This would allow the pathway to be more direct than cutaneous pathways lower down in the spinal cord, although requiring a longer latency. For example, Lundberg and Schomburg(1987) recording from the L6 group II interneurons demonstrated that cutaneous input required at least three synapses to reach the motoneuron and therfore represents a less direct pathway for cutaneous afferents. ie. involves more synapses. It can be seen from this discussion that until we have recorded from interneurons that mediate low threshold cutaneous effects we cannot describe these pathways as being disynaptic although there is some hypothetical evidence that suggests a disynaptic pathway.

Effects from Group Ib Muscle Afferents in Transected Cats

In contrast to the group II effects, synaptic effects from group Ib muscle afferent stimulation produced a different pattern of synaptic effects as to that previously reported in the literature on acute spinal cats. This study examined the Ib pathway from ankle extensors to hamstrings motoneurons of chronic spinal cats . In acute spinal cats, this pathway has been shown to produce substantial excitation in PBST motoneurons. Jankowska et al (1981) reported EPSPs occurring in 94% of PBST motoneurons from stimulation of Ib muscle afferents of ankle extensors. Eccles et al (1957) using acute spinal, phenobarb anesthetized cats reported a lower frequency 24 %, in PBST motoneurons from stimulation of Ib pathways from ankle extensors. Our data in the chronic spinal animal reported the lowest frequency of EPSPs in presumed flexors (13%) (Table III) .

The evidence demonstrates that the effects from stimulation of Ib afferents of the ankle extensors does not occur with as high a frequency as group II effects from quadriceps stimulation, in the same sample of flexor motoneurons. This would suggest that at least this group of Ib interneurons that receive convergence from ankle extensor muscle afferents (Czarkowska et al 1981) do not mediate a powerful low threshold excitatory cutaneous pathway to PBST motoneurons. However, because of the fractionation of input to Ib interneurons (Harrison and Jankowska 1983) it is possible that another combination of Ib muscle afferent pathways could be involved in the transmission of low threshold cutaneous effects.

Comparison Of Lesioned and Unlesioned Preparations

The purpose of this thesis was to determine if there were differences in cutaneous reflex pathways following low threshold stimulation between transected (acute and chronic transection) and intact animals. The difficulty in identifying cutaneous reflexes on the basis of electrical stimulation as well as examining convergence patterns at the motoneuronal level have made it difficult to isolate private localized cutaneous pathways from the more widespread effects of the flexion reflex. However, this initial survey has resulted in several conclusions that can lead to further studies.

Flexors

In the acute and chronic transected cats there is an immediate increase in the excitability following low threshold stimulation of tibial afferents as compared to the unlesioned preparation. In the anesthetized unlesioned animals following tibial stimulation there is one example of an EPSP at 2T but a higher incidence of EPSPs was found when the stimulus strength was raised to 5T. In the transected cat 2T stimulation of tibial afferents resulted in EPSPs in the majority of flexor motoneurons. The mean latency of the earliest EPSPs produced in the transected cats did not differ between acute and chronic preparations. These low threshold excitatory pathways appear to follow the "rules" of the flexion reflex in that they produce excitation of flexors and inhibition of extensors. Further, these flexor motoneurons also receive convergent excitation from group II quadriceps muscle afferents, which together with the excitation from cutaneous afferents is consistent with what the flexion reflex would predict. Because of the strong group II input from quadriceps it was hypothesized that the L4 interneuron may mediate this excitatory pathway which was further characterized as being a disynaptic pathway.

It was also found that the incidence of Ib excitation from Ib muscle afferents is decreased in the chronic spinal animal as compared to the acute transected cat. This pathway was hypothesized as being a possible candidate for localized cutaneous effects. Because there were no differences between the transected animals following tibial stimulation this pathway cannot be involved in transmission of low threshold tibial effects to the PBST motoneurons.

Other cutaneous inputs from SP, Saph and Sur-Perf, also produced a high incidence of excitation in flexors of transected cats. However, they also produced EPSPS in the unlesioned preparations with a comparable frequency. Although not analyzed systematically, comparing the amplitude of EPSPs between unlesioned and transected cats, one can see examples where there might be a tendency for smaller amplitude in the unlesioned preparation (fig 1 and 2 with fig 6 The best example is that from SP stimulation).

Extensors

The effects in extensors have proven to be more difficult to analyze. It appears that electrical stimulation of cutaneous nerves produce variable effects such that we can not predict a consistent pattern of effects. One exception was seen following stimulation of CCF , which produced excitation in 8 out of 8 motoneurons. It was hypothesized that this was a localized excitatory effect similar to that described by Hagbarth(1952) in other cat hindlimb extensors. We have not studied this nerve in the acute cat so we cannot comment on the effects of chronic spinalization.

The other group of cutaneous inputs (Saph and Sur-Perf,) show a tendency to a greater incidence of EPSPs following transection but in contrast to CCF, they also produce a higher incidence of IPSPS or mixed effects.

In contrast to the increased incidence of excitatory effects discussed above, stimulation of the tibial and SP nerves consistently produced inhibition in extensors. Although the number of effects to compare between preparations are small (see Table II), if there is any change following transection with stimulation of these nerves is that there is an increase in the incidence of inhibition. Further, the mean latency from tibial stimulation (2.84 ms) is shorter than that from SP stimulation (4.29 ms).

The changes that occurred following transection in low threshold cutaneous pathways were different between the presumed flexors and extensors of the hamstring muscles. The flexors are more predictable than the extensors in their response to low threshold cutaneous stimulation. However, tibial stimulation, produced the most consistent response between the two muscle groups ie. increased incidence of EPSPS to flexors and IPSPs to extensors, following acute and chronic transection of the spinal cord. This pattern of PSPs would suggest that this pathway is involved in the flexion reflex despite activation at very low thresholds. The similarity of tibial effects in acute and chronic spinal animals implies that the increased reflex excitability when the sole of the foot is stimulated, is due to removal of a tonic descending inhibition rather than a segmental reorganization of afferent input.

Classification of Motoneurons

As outlined in the methods some motoneurons were classified according to their group I input from Quad. After being identified as a hamstring motoneuron antidromically, if the cell received disynaptic inhibition from group I Quad stimulation it was classified as a flexor (PBST). If the hamstring motoneuron received inhibition greater than 1.8 ms it was classified as an extensor (SMAB). Included with the stimulation of quadriceps was the muscle rectus. Rectus is a double joint muscle that results in flexion of the hip and extension of the knee, an opposite action to PBST. Eccles and Lundberg showed in 1958 that rectus produced disynaptic inhibition in all of the hamstring muscle groups, but gives no indication of the incidence of occurrence. He commented on the fact that some hamstring muscle groups particularly SMAB muscles did not receive input from rectus. Subsequently this group of SMAB motoneurons were found to resemble the hip extensor adductor femoris, with regards to group Ia heteronymous connections, and did not receive inhibition from rectus or quadriceps (Eccles and Lundberg 1958) The SMAB motoneuron pool is therfore not a homogenous population with regards to group Ia heteronymous connections.

During locomotion , SMAB motor units have been shown to have a pattern of activity similar to other limb flexors as well as extensors. Engberg and Lundberg(1968) using EMG analysis of locomotion of an unrestrained unlesioned cat, provided evidence that the tibial portion of the semimembranosis behaved like semitendinous (knee flexor) during trot and walk (compare fig 7 to fig 4 Engberg and Lundberg 1968) In the same study, EMG results from the femoral part of semimembranous show a pattern of activity similar to other extensors of the hindlimb. (see fig 2 Engberg and Lundberg 1968) Engberg and Lundberg (1968) comment on the fact that it was not clear whether these motor units from the femoral or tibial section of semimembranosis receives a different pattern of activity to the same motor units or whether flexor activity was evoked in one group of motor units and extensor activity evoked in another. In this study, because we have included rectus we have grouped together muscles of the hamstring group that are homogenous with respect to disynaptic inhibition from quadriceps and rectus (presumed flexors), and isolated them from the muscle groups that may have a more pure hip extensor function similar to adductor femoris. This would mean that some SMAB motoneurons maybe incorrectly classified as presumed flexors. However, as shown in Table

II we have reported a very low incidence of inhibition in presumed flexors, the typical FRA pattern to extensors. Therefore, the incorrectly classified SMAb motoneurons presumably receive a common pattern of excitation from FRA afferents but that we have underestimated its occurrence. Because these extensors receive an FRA input that resembles flexors, possibly during locomotion , flexor activity is being sent to the flexor-like motor units ie. those that receive disynaptic inhibition from Quad, of the semimembranous muscle and extensor activity to the extensor-like motor units of the semimembranous muscle. To test this hypothesis experiments would have to be done to see if SMAB motoneurons that receive disynaptic inhibition from Quad and receive excitation from cutaneous afferents is active with other hindlimb flexors during the gait cycle.

It is also worth noting that the SMAB motoneurons in acute spinal animals all received inhibitory input from cutaneous stimulation. In contrast AB motoneurons could be flexor-like or extensor-like in chronic spinal animals. For example, AB motoneurons in the chronic spinal cat that received disynaptic inhibition from Quad received a common excitatory input resembling other flexors. Those AB motoneurons classified with the presumed extensors acted like the other extensors receiving a variable pattern of inputs. Because only inhibitory cutaneous effects were found in the SMAB cells of the acute spinal animal, it could be possible that the chronic spinal cat is more stable because of the time following transection which has allowed the flexor-like effects to be seen in the AB motoneurons. It is interesting to note that these discrepancies are in extensors, the muscle groups that appear to be affected more by spinal shock. (Sherrington 1910, Creed et al 1932). In contrast to the extensors, the flexors , muscle groups that are more resistant to spinal shock, that were misclassified as extensors received only flexor-like excitation from the majority of cutaneous afferents except in one example.

In defense of the criteria used to identify motoneurones in this thesis it appears from the above discussion that no one system of classification is without exceptions. Sherrington defined muscle action according to the flexion reflex behavior. In his preparation both femoral and tibial portions of the semimembranous muscle were inhibited and therefore called extensors (Sherrington 1910). However, during the gait cycle EMG analysis revealed that the tibial portion behaved as a flexor (Engberg and Lundberg 1969). Therfore, based on the muscles response in locomotion the tibial portion of semimembranous could be described as a flexor. In this data cells that received disynaptic inhibition from quadriceps group I stimulation (presumed flexors) received excitation from cutaneous afferents, with four exceptions three of which received excitation in spite of the incorrect classification. However, extensors (SMAB) that received disynaptic inhibition from Quad, received only inhibitory cutaneous input characteristic of extensors in the acute spinal animal. In contrast other extensors (AB) receive flexor like excitatory input but in chronic preparations. Therfore, no one system of classification can identify muscle function. From the differences seen between SMAB motoneurons in the acute and chronic preparations described in this study it appears that acute transection of the spinal cord may effect how a muscle is classified.

Figure 1: Synaptic potentials recorded from an acute spinal cord transected cat, in a PBST motoneuron identified antidromically. This cell was also classified according to group I input from quadriceps as a flexor motoneuron (panel A). This figure demonstrates low threshold excitation from Tib (panel C), Saph(panel E), and Sur-Perf (panel G) that grows with higher thresholds (panel D,F,H,). Note also the group II EPSP at approximately 3 ms from quadriceps stimulation classified by the large change in amplitude of the EPSP between 2T and 5T stimulation (panel A and B).

The upper traces in each panel represent the intracellular records, recorded from lumbar alpha motoneurons. The bottom trace represents the recording from the cord dorsum, a silver ball electrode located on the junction of the dorsum of the spinal cord with the L7 dorsal roots. The cord dorsum was used to indicate the arrival of the peripheral afferent volley to the spinal cord. The nerve stimulated as well as the threshold for stimulation is located in the right corner of each panel. A calibration pulse 2ms wide and 2 mv high is in the far left hand corner of each intracellular trace. All of these conventions will be adhered to in the following figures unless otherwise stated.

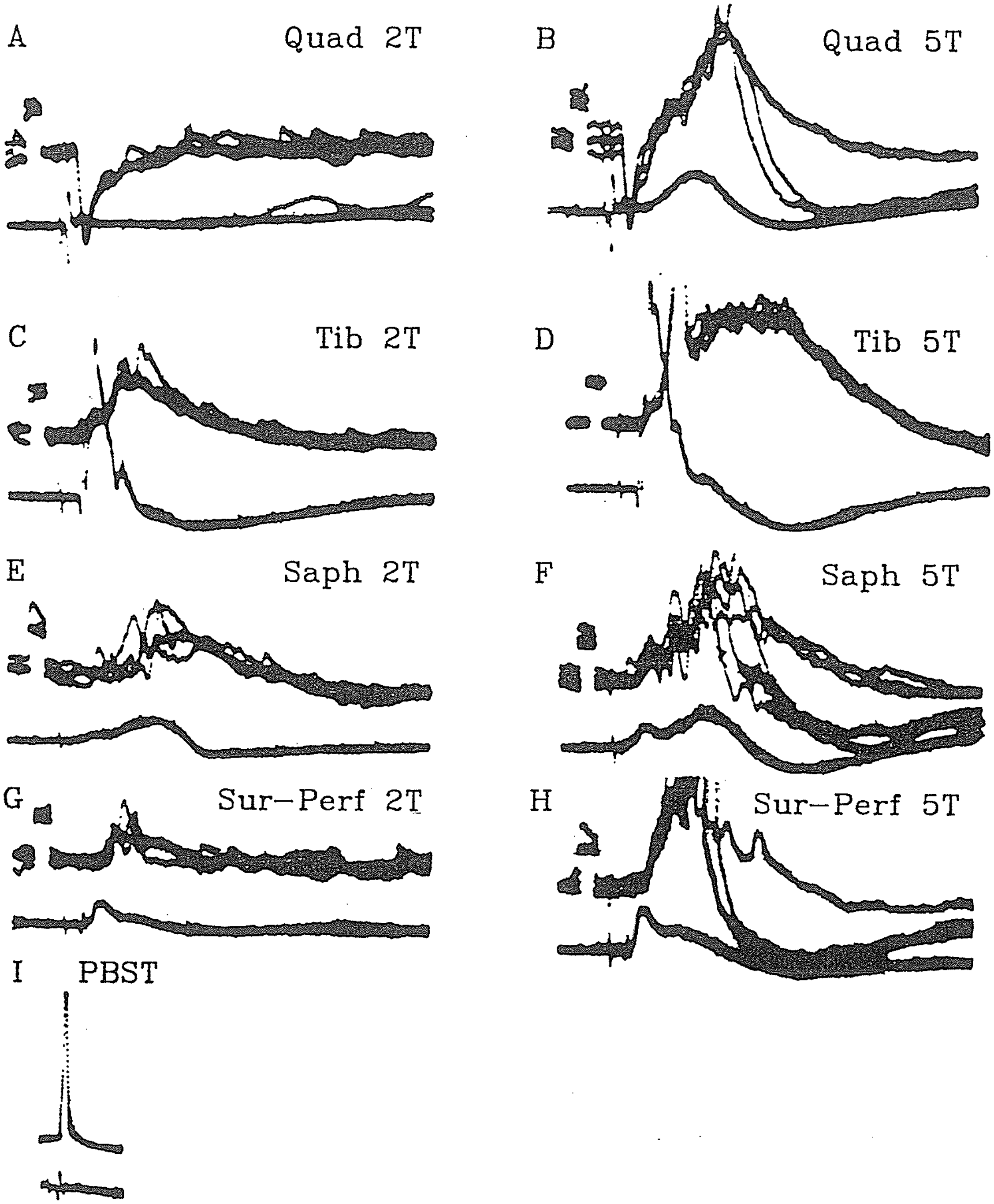
A
Quad 2T
B
Quad 5T
C
Tib 2T
D
Tib 5T
E
Saph 2T
F
Saph 5T
G
Sur-Perf 2T
H
Sur-Perf 5T
I
PBST

Figure 2: Synaptic potentials recorded in a motoneuron innervating the hamstrings in a chronic spinal cat. This motoneuron was classified as a flexor based on its disynaptic group I inhibition from quad nerve stimulation(panel A). This figure demonstrates the finding that in presumed flexor motoneurons most cutaneous inputs are excitatory, and that the amplitude of these EPSPs grow when the stimulus strength is increased from two to five times threshold. Note the smaller effects from CCF (panel I) and Saph (panel M)., see text re discussion of localized effects (Hagbarth 1952). Sp stimulation at 2T (panel O) produced an action potential in this cell. The complete spike was not shown in the figure.

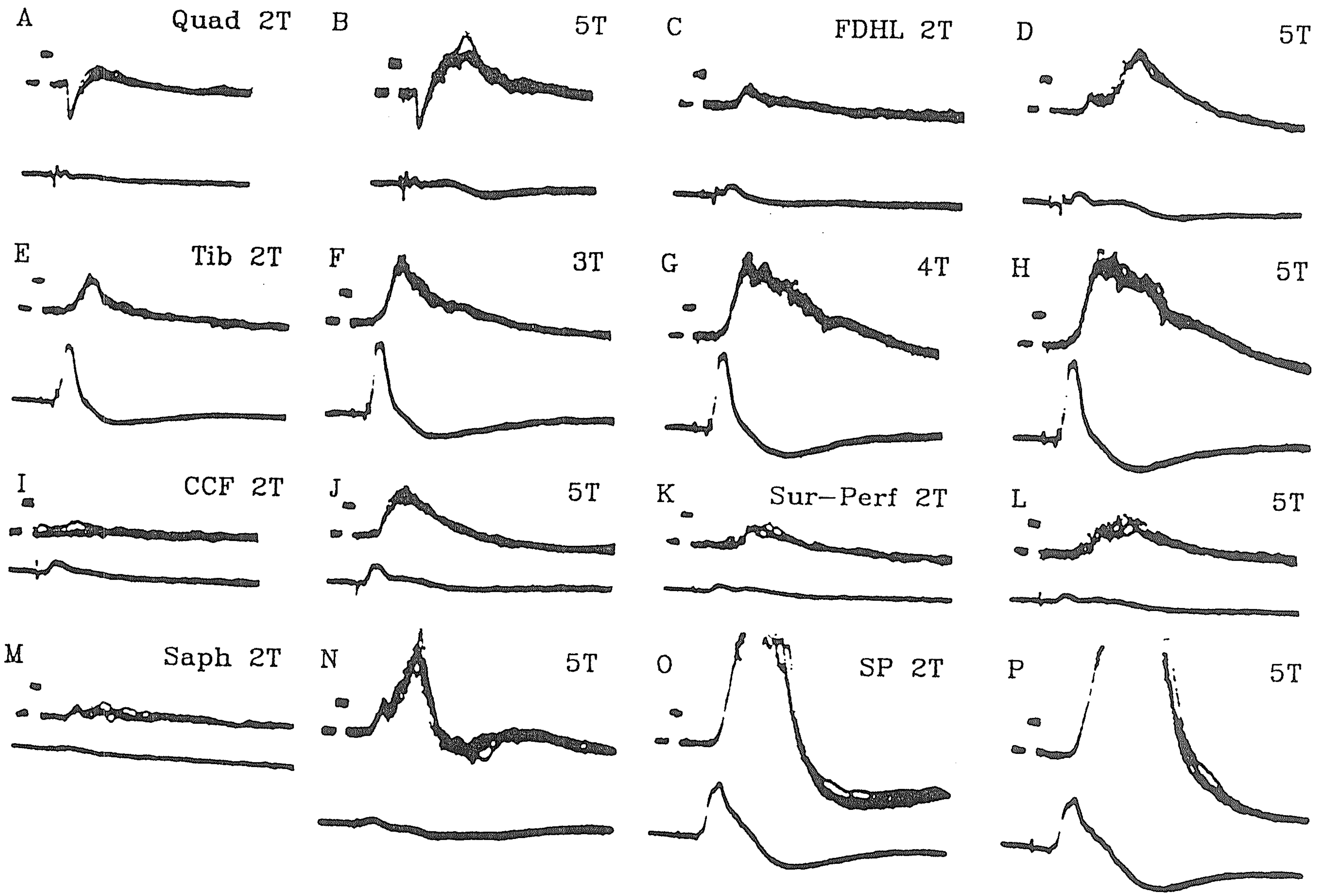
A
Quad 2T
B
5T
C
FDHL 2T
D
5T
E
Tib 2T
F
3T
G
4T
H
5T
I
CCF 2T
J
5T
K
Sur—Perf 2T
L
5T
M
Saph 2T
N
5T
O
SP 2T
P
5T

Figure 3: Synaptic potentials recorded in a Sm motoneuron, from a chronic spinal cord transected cat, identified by antidromic stimulation. This motoneuron was classified as a presumed flexor as it receives disynaptic inhibition from quadriceps stimulation at 1.3T (panel K). This figure demonstrates low threshold excitation from Tib (panel A), Saph(panel D), Perf(panel E), Sural (panel F), Joint (panel I), and SP (panel L). Higher threshold stimulation of muscle afferents (panel H) and cutaneous afferents (panel C) also produces excitation.

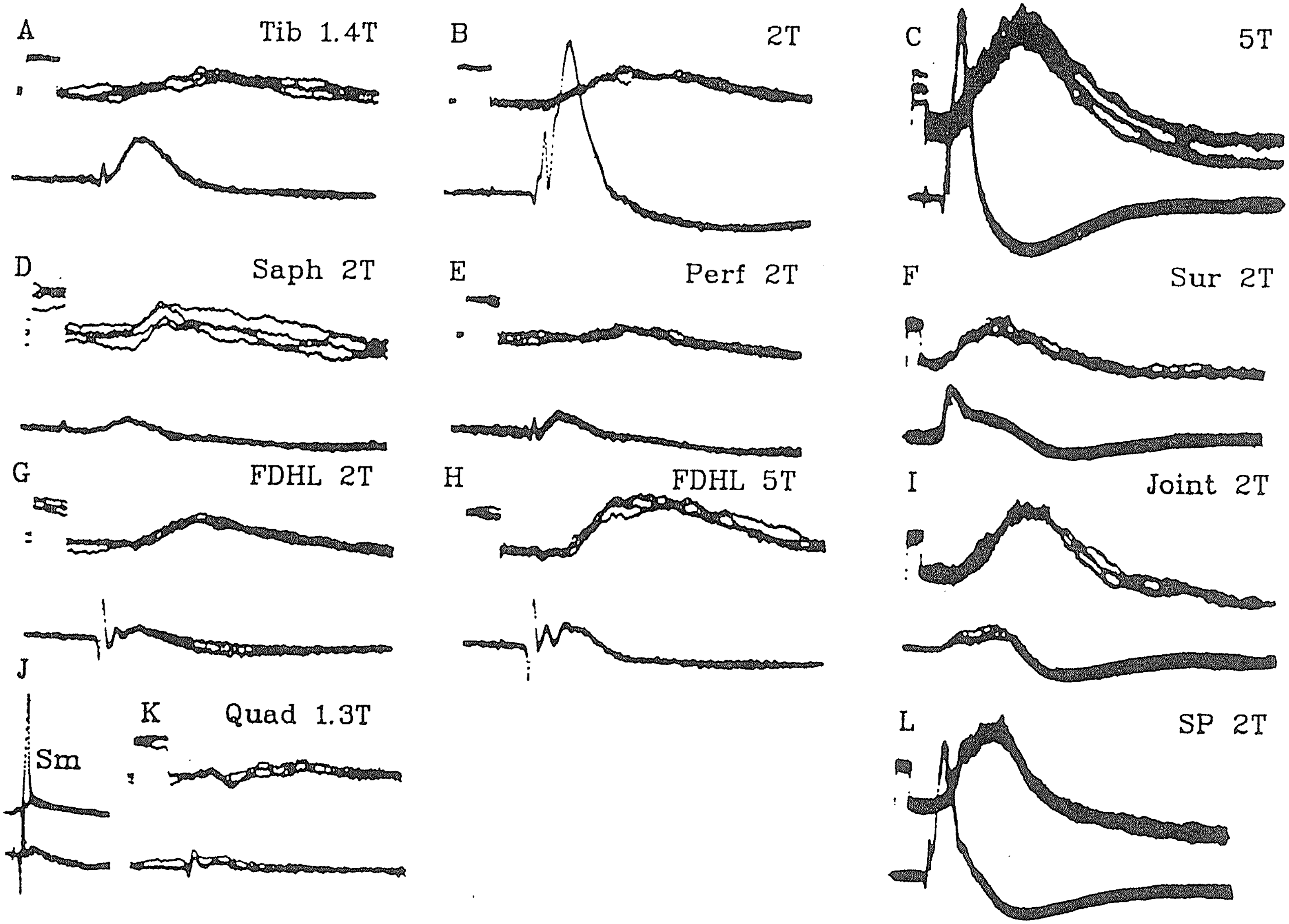
A
Tib 1.4T
B
2T
C
5T
D
Saph 2T
E
Perf 2T
F
Sur 2T
G
FDHL 2T
H
FDHL 5T
I
Joint 2T
J
Sm
K
Quad 1.3T
L
SP 2T

Figure 4: Synaptic potentials recorded from an AB motoneuron in a chronic spinal cat identified on the basis of group I input from quadriceps (including rectus)(panel E). Note that the cell also receives disynaptic inhibition from quadriceps.(panel E) This figure demonstrates a low threshold excitatory pathway from afferents innervating a large receptive field (panels A-H) in a "presumed flexor" motoneuron. This motoneuron illustrates the difficulty in predicting cutaneous effects based on Sherrington's classification of motoneurons. ie.the cell receives input from muscle and cutaneous afferents that is flexor-like, but is identified antidromically as an extensor. Note the mixed PSP from Saph at twice threshold.

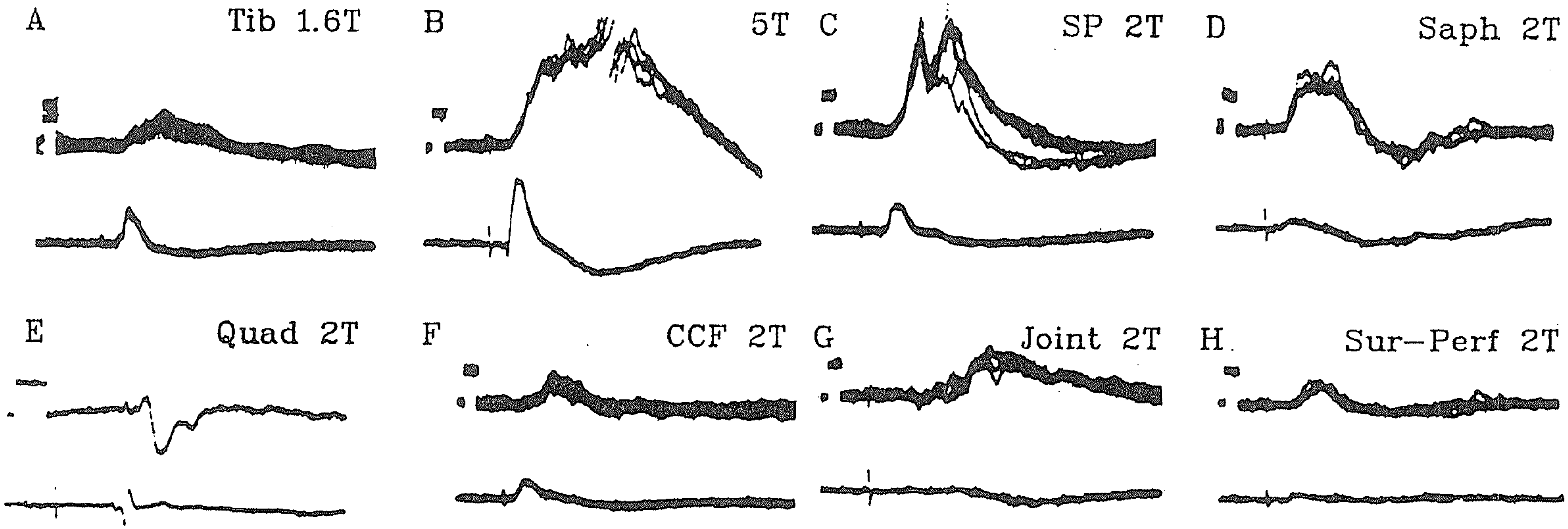A
Tib 1.6T
B
5T
C
SP 2T
D
Saph 2T
E
Quad 2T
F
CCF 2T
G
Joint 2T
H
Sur—Perf 2T

Figure 5: Synaptic potentials recorded in a chronic spinal cat from a hamstring motoneuron that was classified as a presumed extensor cell based on its input from quadriceps at 2T(panel A).Note the monosynaptic heteronymous excitation from Quad stimulation at 2T (panel A) which further identifies this cell as an AB motoneuron. Stimulation of quadriceps at 5T produced a mixed effect in panel B. The middle trace in this panel is an extracellular record from outside the motoneuron following 5T stimulation of Quad. Cutaneous nerves tested produced excitation (Sural panel D or CCF panel E), inhibition (Tib panel I-K, joint panel C, or SP panel G-H) as well as a mixed PSP (Saph panel F). This figure demonstrates the variable effects from cutaneous afferents in a presumed extensor motoneuron. Panels M-P demonstrate a group I (including Renshaw inhibition) from MG stimulation. High threshold stimulation produced different effects from the muscle afferents Quad (panel B) as compared to MG(panel P). Note also the similarity in the shape of the PSPs from Tib, Quad and SP at 5T.

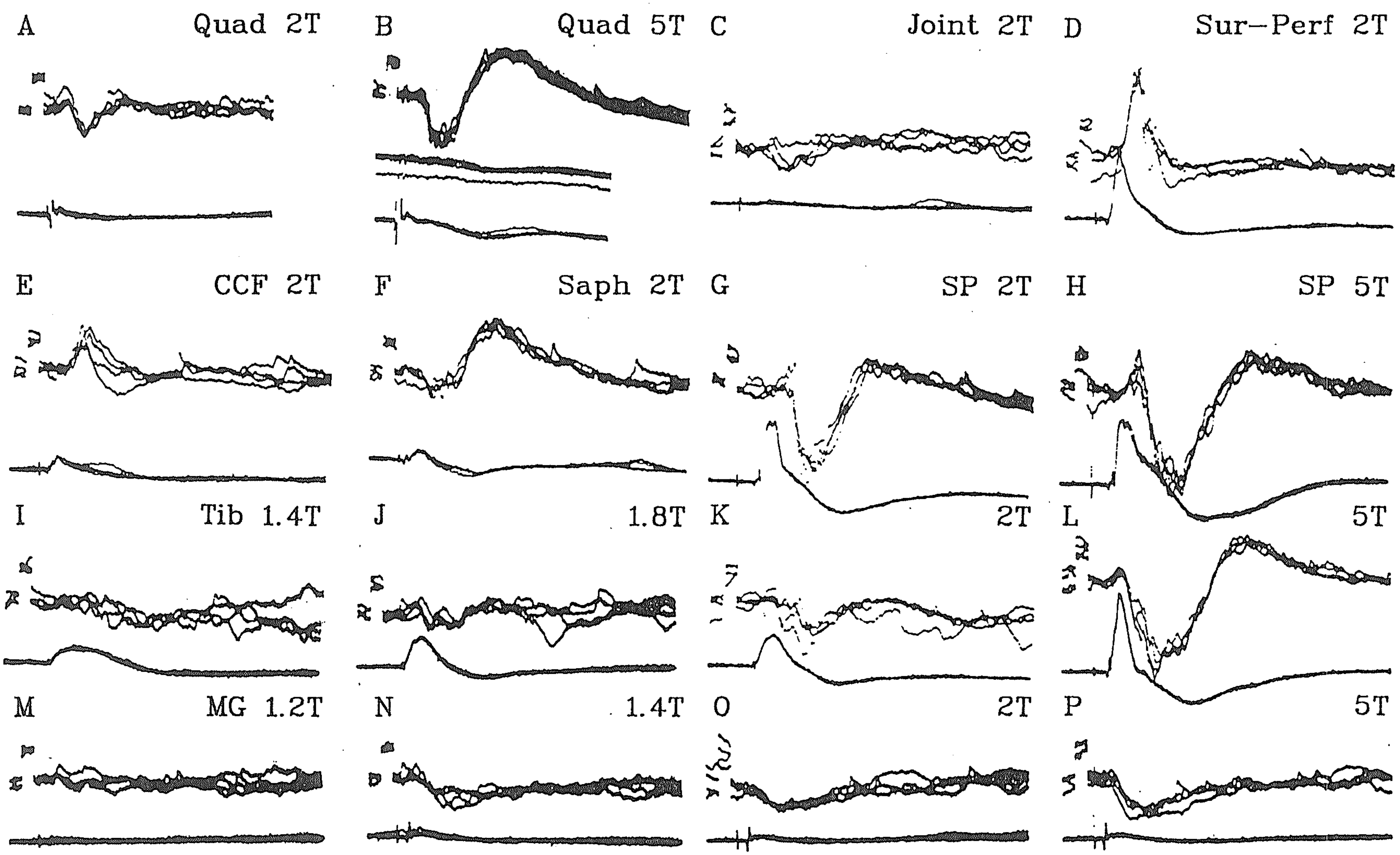
A
Quad 2T
B
Quad 5T
C
Joint 2T
D
Sur–Perf 2T
E
CCF 2T
F
Saph 2T
G
SP 2T
H
SP 5T
I
Tib 1.4T
J
1.8T
K
2T
L
5T
M
MG 1.2T
N
1.4T
O
2T
P
5T

Figure 6: Synaptic potentials recorded in a spinal cord intact, anesthetized cat in a hamstring motoneuron classified as a flexor cell based on its group I input from quadriceps stimulation.(panel G) Cutaneous stimulation resulted in small amplitude PSPs which were excitatory from SP, Sural and Joint(panel D-f) and mixed PSPs from CCF AND Saph(panel B-C). Tibial stimulation (panel A-G) also produced lower amplitude PSPs at 2T, which grew in amplitude with higher thresholds.This figure demonstrates the uncharacteristic finding of excitation in a flexor motoneuron in a spinal cord intact cat that appears to require a higher threshold to produce a PSP that is similar to that found in the chronic cat. (compare panel C fig 1, to fig 2 panel E, to fig 6 panel A)

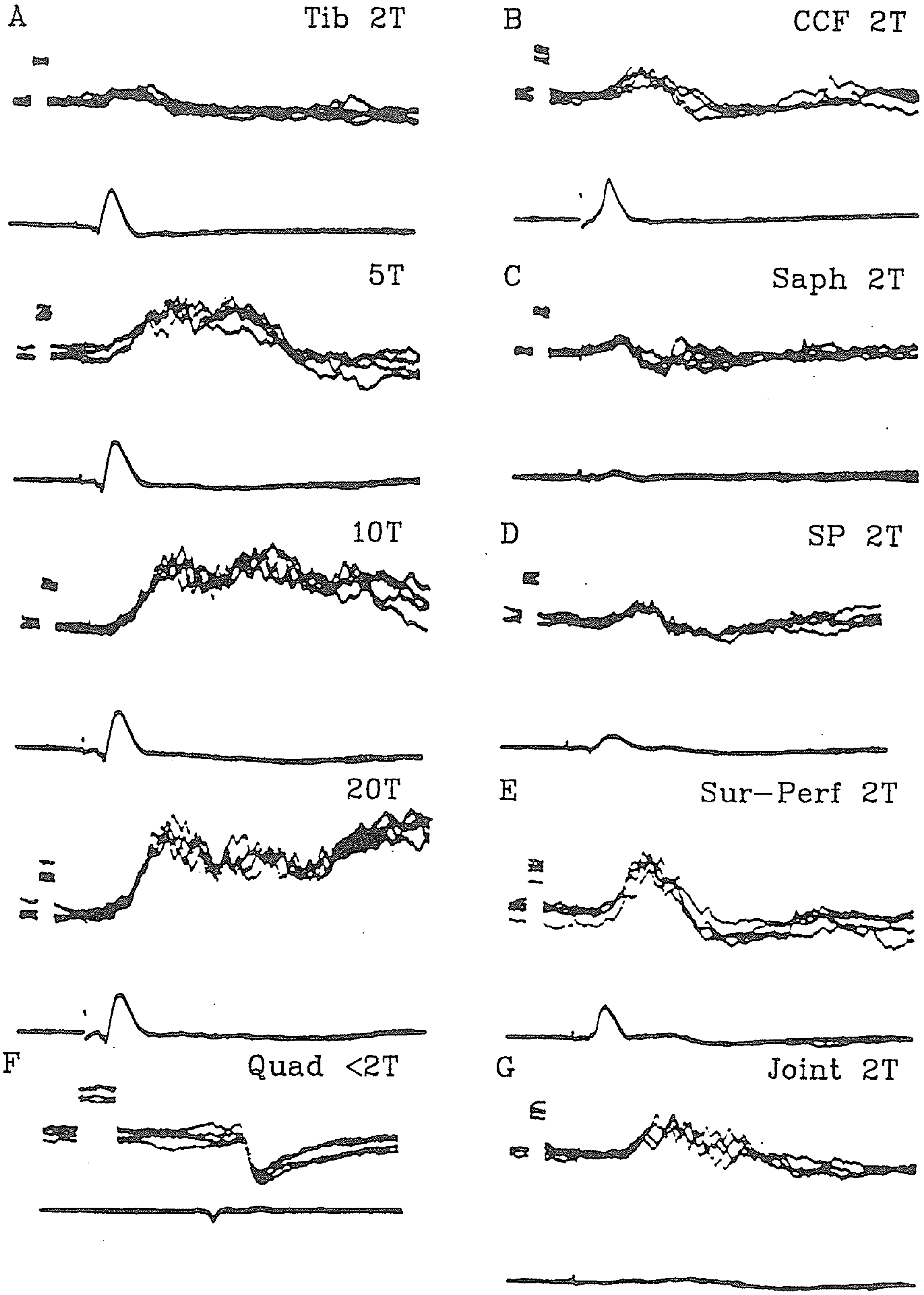
A
Tib 2T
5T
10T
20T
B
CCF 2T
C
Saph 2T
D
SP 2T
E
Sur–Perf 2T
F
Quad <2T
G
Joint 2T

Figure 7: Synaptic potentials recorded in a PB motoneuron in a chronic spinal cat. Panel A is an EPSP resulting from stimulation at 1.25T of the Tibial nerve (in a cuff electrode). Panel B shows an EPSP with the same latency but following electrical stimulation of the central pad of the foot at < 2T. This figure demonstrates the similarity in the PSPS in PB motoneurons from stimulation of cutaneous afferents (B) vs. both motor and sensory afferents(A). Note that panel B, upper trace, is the intracellular recording, lower trace is the cord dorsum. Calibration pulse is the same for both panels A and B.

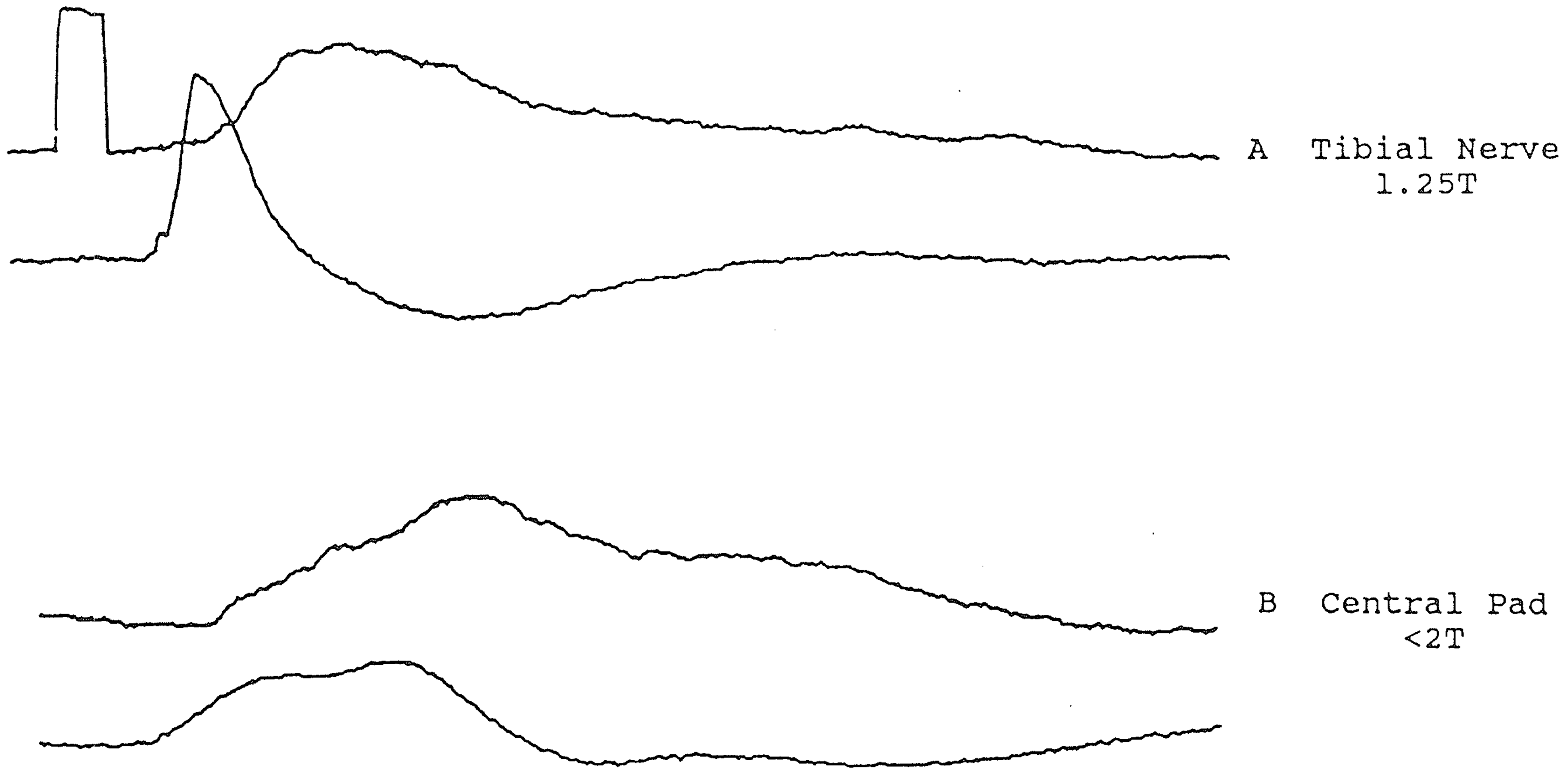
A Tibial Nerve
1.25T
B Central Pad
<2T

TABLE I

The following table represents the means and earliest latencies of PSPs in presumed flexors of the hamstring muscle group. Flexor classification in acute transected animals was by antidromic stimulation from PBST or SMAB as well from group I effects from quadriceps stimulation (see methods.) In chronic transected cats as well as unlesioned cats, flexors were identified on the basis of group I effects from quadriceps stimulation and antidromic identification of hamstrings (Both PBST and SMAb were on the electrode.) Note that the IPSP/Mixed effects in the acute transected data are all from antidromically identified SMAB motoneurons.

bracketed data was tested with t-test but not significant
*significant at $p=.01$
** significant at $p=.005$

TABLE I

INCIDENCE AND LATENCY OF CUTANEOUS EFFECTS IN PRESUMED FLEXORS

		TOTAL	NE	EPSPs	IPSPs	MIXED
TIB	unlesioned	14	8	2.00 ±1.06 (2)	4.42 ±1.82 (3)	1.50 (1)
	chronic sp	31	3	2.72 ±.86 (26)	2.47 ±.52 (2)	(0)
	acute sp	17	0	2.62 ±.40 (12)	2.25 +.29 (4)	2.00 (1)
CCF	unlesioned	8	5	2.80 (1)	(0)	2.40 ±.53 (2)
	chronic sp	16	4	2.28 ±.57 (11)	6.00 (1)	(0)
SP	unlesioned	8	1	2.94 ±.58 (6)	(0)	3.00 (1)
	chronic sp	29	1	2.38 ±.37 (27)	3.20 (1)	(0)
SAPH	unlesioned	10	0	2.27 ±.56 (7)	5.25 ±.35 (2)	2.50 (1)
	chronic sp	24	3 **	2.09 ±.39 (15)	(2)	2.00 ±0.0 (4)
	acute sp	14	1	3.25 ±.63 (10)	2.67 ±.29 (3)	(0)
SUR PERF	unlesioned	9	1	2.42 ±.43 (5)	(0)	3.33 ±.35 (3)
	chronic sp	20	1 *	2.30 ±.73 (16)	3.60 ±.57 (2)	2.50 (1)
	acute sp	13	0	2.98 ±.35 (10) *	(0)	3.00 ±0.0 (3)

TABLE II

The following table represents the means and earliest latencies of PSPs in presumed extensors of the hamstring muscle group. Extensor classification in acute transected animals was by antidromic stimulation from SMAB as well as the lack of group I disynaptic inhibition from quadriceps (see methods).

In chronic transected cats as well as unlesioned animals extensors were identified on the basis of the lack of disynaptic inhibition from quadriceps as well as the antidromic identification from hamstrings (Both SMAB and PBST were on the electrode)

TABLE II

INCIDENCE AND LATENCY OF CUTANEOUS EFFECTS IN PRESUMED EXTENSORS

		TOTAL	NE	EPSPs	IPSPs	MIXED
TIB	unlesioned	6	2	4.00 (1)	2.90 ±.17 (3)	(0)
	chronic sp	28	2	1.75 ±.35 (2)	2.84 ±.69 (20)	3.02 ±.56 (4)
	acute sp	5	0	(0)	2.82 +.18 (5)	(0)
CCF	unlesioned	6	1	2.00 (1)	4.58 ±1.00 (2)	2.00 ±0.0 (2)
	chronic sp	8	0	2.36 ±.37 (8)	(0)	(0)
SP	unlesioned	6	0	2.95 ±.78 (2)	5.00 (1)	3.78 ±1.53 (3)
	chronic sp	17	1	2.66 ±.47 (5)	4.29 ±1.18 (8)	3.13 ±.81 (3)
SAPH	unlesioned	7	0	(0)	4.76 ±1.08 (6)	2.50 (1)
	chronic sp	18	1	3.16 ±.48 (5)	3.96 ±.89 (7)	3.60 ±.76 (5)
SUR PERF	unlesioned	5	1	2.80 (1)	4.59 ±.87 (2)	2.00 (1)
	chronic sp	10	0	2.18 ±.36 (5)	4.50 (1)	2.37 ±.41 (4)

TABLE III

The following table represents effects from muscle afferents following stimulation at group I strength and group II stength for ankle extensors (MG and LG) as well as quadriceps. (see methods)

TABLE III

CHRONIC SPINAL HAMSTRING MOTONEURONS

		TOTAL	NE	EPSPs	IPSPs*
FLEXORS	MG GROUP I	17	12	1	4
	LG-S GROUP I	12	8	3	1
	MG GROUP II	11	10	1	0
	LG-S GROUP II	13	5	7	1
	QUAD GROUP II	12	1	10	0
EXTENSORS	MG GROUP I	5	3	0	2
	LG-S GROUP I	3	2	0	1
	MG GROUP II	1		0	1
	LG-S GROUP II	3		0	3
	QUAD GROUP II	8		0	8**

* may include renshaw cell mediated inhibiton as ventral roots were intact

** IPSP or mixed effect ie. IPSP followed by an EPSP

LIST of ABBREVIATIONS

AB	Anterior Biceps
CCF	Cutaneous Caudo-Femoralis
C.P.	Common Peroneal
EPSP	excitatory post synaptic potential
FDHL	Flexor Digitorum and Flexor Hallucis Longus
IPSP	inhibitory post synaptic potential
LG-S	Lateral Gastrocnemius and Soleus
MG	Medial Gastrocnemius
ms	millisecond (s)
Perf	Perforant
Plant	Plantaris
PSP	post synaptic potential
PB	Posterior Biceps
Quad	Quadriceps
Saph	Saphenous
SM	Semimembranous
SMAB	Semimembranous and Anterior Biceps
ST	Semitendinous
Sur-Perf	Sural and Perforant
SP	Superificial Peroneal
T	Threshold
Tib	Tibial

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