A comparison of the flexion reflex in decerebrate vs. spinal cats
by Ward Allen Yuhas

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
The flexion reflex (FR) is a spinal reflex which serves as a protective mechanism by withdrawing a limb from a noxious stimulus. The motor response of the FR consists of an early and a late component. The early component is thought to be due to impulses from A-delta fibers, and the late component is believed to be the result of C fibers, both relaying impulses from noxious stimuli.

It is known that the excitability of the FR increases when the spinal cord is cut, which indicates that "supraspinal" centers are inhibitory on this reflex. These descending pathways preferentially inhibit the responses of spinal interneurons to C fiber inputs more than to A-delta inputs. Therefore, it seems probable that eliminating inhibition on the FR by cutting the spinal cord would result in a greater contribution of these C fibers to the reflex. The results of this study demonstrate just the opposite of this hypothesis.

Excitability of the FR was tested first in decerebrate cats to evaluate the reflex under the tonic descending inhibitory influence from the brainstem. The FR was evoked by electrically stimulating the tibial nerve at both low and high frequencies, and was monitored by recording isometric tension from the semitendinosus muscle from the ipsilateral limb. The spinal cord was then transected at the thoracic level and the tests were repeated.

The threshold intensity required to evoke the FR was significantly lower in the spinal preparation compared to the decerebrate condition, indicating general increased responsiveness. Other indices of the FR excitability supported this observation, including a decrease in the latency of the response and the time required to reach peak amplitude. More importantly, the reflex response diminished much more quickly, showing no late component of the reflex. Collectively, these data suggest that A-delta input to the FR is enhanced, and that of C fiber input is diminished after spinal transection.

These results indicate that our understanding of the FR and spinal reflex mechanisms in general is inaccurate, especially regarding the modulation of these reflexes by supraspinal centers. Furthermore, since reflexes such as the FR are used as animal models of pain perception, these results raise doubts about the validity of using the FR to interpret pain sensation.

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Ward Allen Yuhas

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Signed Ward A. Yukas Date 9/16/91

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ABSTRACT

The flexion reflex (FR) is a spinal reflex which serves as a protective mechanism by withdrawing a limb from a noxious stimulus. The motor response of the FR consists of an early and a late component. The early component is thought to be due to impulses from A-delta fibers, and the late component is believed to be the result of C fibers, both relaying impulses from noxious stimuli.

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These results indicate that our understanding of the FR and spinal reflex mechanisms in general is inaccurate, especially regarding the modulation of these reflexes by supraspinal centers. Furthermore, since reflexes such as the FR are used as animal models of pain perception, these results raise doubts about the validity of using the FR to interpret pain sensation.

INTRODUCTION

The control of movement is essential for the survival of all organisms, especially if the movement is in response to a potentially harmful stimulus. Although higher animals are capable of some very complex motor skills, many of which require conscious input, some movements are automatic and involuntary. Some of the simplest of involuntary movements are reactions to stimuli from the environment that elicit rapid and predictable motor responses: we call these movements reflexes. Many of the reflexes serve to protect an organism from noxious stimuli encountered in the environment, such as excessive heat or a sharp object. The **"flexion reflex" (FR)** is one such reflex. Also termed the "withdrawal reflex", the FR functions to withdraw a limb from noxious stimuli. In humans this basic reflex response to noxious stimuli is normally associated with the conscious perception of pain. In animals the reflex can be easily demonstrated, and although we obviously cannot obtain subjective comments about pain, the FR in animals at least provides us with a model with which to investigate the relationship between sensory stimuli and motor responses.

The first comprehensive studies of the FR began near the turn of the century (Sherrington, 1906, 1910). Sherrington provided a solid anatomical as well as physiological

foundation for this and other spinal reflexes, and all current investigation into the FR relies on the decades of study contributed by Sherrington and his colleagues. Since this pioneering work, investigators have attempted to identify the specific elements that comprise the circuitry of the FR, and also the ways in which this spinal reflex might be modified in the central nervous system (CNS). In this reading I will discuss the afferent contribution conveying sensory information to the spinal cord, the interneurons in the CNS that relay these impulses, the motor response of the FR and also descending tracts from supraspinal centers that influence the FR.

Afferent inputs to the spinal cord are composed of several different fiber types, each conveying specific information to the central nervous system. We know also that certain areas of the brain send projections to the spinal cord to modify responses of spinal cord interneurons to incoming sensory information. In this study the contributions of two different (A and C) fiber inputs to the FR were examined in both decerebrate and spinal cats in order to better understand how supraspinal centers differentially affect the motor responses to these inputs.

Literature Overview

The simplest neural arc of all spinal reflexes is illustrated by the monosynaptic stretch reflex. In this

circuit, the Ia afferents innervating muscle spindles synapse directly onto the motorneurons that send their axons to muscle. The FR, however, is a polysynaptic reflex, which means that impulses directed to the dorsal spinal cord must relay through one or more interneurons before affecting the motorneurons in the ventral horn of the spinal cord grey matter. There is known to be a great deal of afferent convergence onto the interneurons in the dorsal horn from afferents, collateral afferent branches from other spinal cord segments and descending tracts from brain centers (Kolmodin and Skoglund, 1960; Wall, 1967). This convergence of afferent and descending input allows for a great deal of modification of incoming signals before motorneurons are activated.

Primary Afferent Input to the FR

Several different types of axons transmit impulses from skin and muscles to the spinal cord (Erlanger and Gasser, 1937; Lloyd, 1943). Table 1 lists current classifications of cutaneous and muscle/tendon fiber types based on size and conduction velocity.

All A-type cutaneous and Groups I-III muscle/tendon fibers are myelinated, which explains their faster conduction velocities. Most of these fibers innervate mechanoreceptors (skin) or proprioceptors (muscle), or, as in the case of many A-delta fibers, may be found in the skin as "free nerve endings" which respond primarily to noxious stimuli. Cutaneous C and Group IV muscle fibers are nonmyelinated

axons, which accounts for their slower conduction velocities. Most of these fibers also have free nerve endings that respond to noxious thermal, chemical and mechanical stimuli, though some of these endings do respond to innocuous sensations of the same modalities.

Table 1. Afferent axon classifications. (from Patton et al., 1989)

Cutaneous Axons	Muscle/Tendon Axons	Axon Diameter	Conduction Velocity
A-alpha	Group I	13-20 um	80-120 m/s
A-beta	Group II	6-12 um	35-75 m/s
A-delta	Group III	1-5 um	5-30 m/s
C	Group IV	0.2-1.5 um	0.5-2.0 m/s

The fiber types that contribute to the FR were collectively termed the "flexor reflex afferents" (FRA) by Eccles and Lundberg (1959), and include high and low threshold afferents from skin and high threshold muscle and joint afferents (groups II, III and IV). Since the FRA category contains both cutaneous and muscle/joint afferents, it can be somewhat confusing to interpret. Because of this, there is not universal acceptance of this classification. Boivie and Perl (1975) state, "(the FRA) designation ...suggests an unproven common function for a mixed population of sensory elements and may do more to confound than to clarify issues."

The A and C classification of cutaneous afferents based on size and conduction velocity was introduced by Erlanger and Gasser (1937), and this classification is widely used in current research dealing with nociception (see Taylor and Pierau, 1991). Considering that a natural stimulus to elicit the FR would likely be encountered at some peripheral receptive field of the skin, it makes sense to examine the FR based on the cutaneous grouping.

Once different afferents to the FR were described, a logical query was how these inputs relate to different pain sensations. The painful sensation of stubbing a toe has undoubtedly been experienced by us all, and individual descriptions of the pain encountered would very likely be similar: an intense, stinging pain is first perceived, followed a second or so later by dull, throbbing pain that persists. Knowing that A fibers conduct their signals to the spinal cord more quickly than do C fibers, investigators logically attempted to associate the "first" (stinging) and "second" (throbbing) pain sensations in humans to A and C fiber inputs, respectively. Zotterman (1939) was one of the first to suggest that the two perceptions of pain are carried in A and C fibers, and this assertion was later supported by several other investigators (see Price, 1972 for references).

Not only has sensation been found to exhibit a duality, but motor responses of the FR also have two components. When a cutaneous nerve is electrically stimulated at supramaximal

intensity (so that all afferents are activated), a flexion reflex will ensue. The response of a flexor muscle to this input is not a simple contraction, however. The recorded FR will illustrate two peaks, one with a relatively short latency and of short duration, and one with a longer latency and extended duration. One explanation of these two different reflex components suggested that the long latency peak was a result of some FRAs entering the spinal cord and encountering a "central delay" (Anden et al., 1964). A later theory proposed by Dimitrijevic and Nathan (1970) stated that these short and long latency components of the FR were the results of A-delta fiber and C-fiber inputs, respectively.

In summary, the A and C fiber afferents can be related to: (i) the characteristic first and second pain sensations, respectively, and (ii) the respective early and late components of the FR. It seems, then, that the early and late components of the FR could be motor responses that correspond directly with the first and second pain perceived. Although this idea has yet to be proven, studies of the interneuronal elements that connect sensation to motor output have provided more evidence for the notion. Price (1972) has shown that interneurons located in the dorsal horn of the spinal cord grey matter (discussed below) respond to both A and C fiber inputs; these responses were consistent with (i) first and second pain responses in humans and (ii) the FR recorded from cats (Price, 1972). Several other investigators have

demonstrated a relationship between nociception and the FR recorded in human subjects (Kugelberg, 1948; Dimitrijevic and Nathan, 1970; Willer et al., 1979; McMillan and Moudy, 1986).

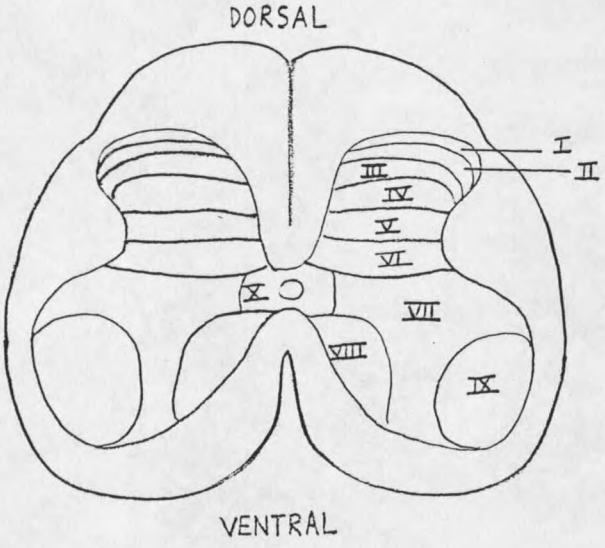
Central Projections of Afferents

Determining the functional connectivity of the FR requires a thorough knowledge of the anatomy of the spinal cord. Realizing a lack of consistent interpretations of spinal cord organization, Rexed (1952) defined a laminar arrangement of the spinal grey matter based on cytoarchitectural differences: i.e., based on the sizes and shapes of spinal cord cell bodies as well as the density and distribution of these somata. The dorsal horn of the grey matter in particular can be divided into several layers based on cell types and densities (laminae I-VI), but the ventral horn is more "grouped" than layered, containing most notably the columns of motoneuron cell bodies in lamina IX (Figure 1).

One of the first distinctive features of the spinal cord grey matter to be characterized was that of the substantia gelatinosa (reviewed in Light et al., 1979), which corresponds to Rexed's lamina II. This region was suspected to be involved with pain sensation, based on the finding that small diameter fibers project to this area (Ranson, 1915). This theory was given support in subsequent years when fiber types were related to pain sensation in humans, and again when it was confirmed that small diameter afferents that respond to noxious stimuli enter the substantia gelatinosa and

neighboring marginal zone, or Rexed's lamina I (Earle, 1952). Using simple Golgi staining techniques, Rethelyi (1977) also traced small unmyelinated afferents to their termination in the substantia gelatinosa.

Figure 1. A cross-sectional diagram of the cat spinal cord near the upper lumbar level. Roman numerals indicate the ten laminae proposed by Rexed (1952).



The introduction of techniques such as axon labeling with dyes, autoradiography and electron microscopy allowed investigators to more accurately describe the projections of afferent fibers to the spinal cord. Labeling of afferents to the dorsal horn with horseradish peroxidase led to the discovery that "thin" fibers terminate in the more superficial zones of laminae I and II, and that "thick" fibers terminate deeper in the dorsal horn (Light and Perl, 1977, 1979). Substantial evidence has been gathered by several other groups

using various techniques to indicate that small myelinated nociceptive (A-delta) afferents terminate in lamina I, and that unmyelinated nociceptive afferents terminate in lamina II (LaMotte, 1977; Kumazawa and Perl, 1978; Light et al., 1979; Ralston and Ralston, 1979).

Considerable attention has been given to the dorsal horn laminae, in part because of a theory of pain modulation proposed by Melzack and Wall (1965). They suggested that the substantia gelatinosa works as a "gate control system" that checks incoming pain signals from peripheral fibers. This attempt to identify a mechanism that traffics pain sensation directed attention to some very important concepts about sensory integration (see Rethelyi and Szentagothai, 1973), including (i) convergence of afferent input, (ii) interactions of spinal cord cells responding to different inputs, and (iii) descending modulation from brain centers.

Generally, convergence increases as one descends through deeper layers of the dorsal horn, due to each cell receiving many afferent inputs. Cells that respond to several inputs often discharge in response to different modalities of noxious skin sensation (C-polymodal) as well as to different innocuous mechanical stimuli, and thus have been termed "multireceptive". Physiological evidence for this was provided by Wall (1967), who recorded from cells in laminae IV, V and VI that responded to several different types of cutaneous input from both A and C fibers. Wall also noticed

that cells in deeper laminae had larger receptive fields than those in more superficial laminae, which indicates that these cells may have a greater number of inputs.

Christensen and Perl (1970) more accurately identified cells in laminae I and II, and discovered that the lamina I cells respond to strong mechanical stimulation of skin, and therefore probably receive input from A-delta fibers; in contrast, lamina II cells respond to both strong mechanical stimuli and noxious heat. Thus, even in the most superficial layers of the dorsal horn there is convergence of different afferent input. Light et al. (1979) confirmed the cell types of laminae I and II that respond to A-delta and C fiber input, respectively, using a combination of electrical stimulation and horseradish peroxidase labeling. Cells have also been discovered in these laminae that habituate to afferent inputs or respond with prolonged discharges (Wall et al., 1979; Fitzgerald, 1981).

Not only is the cytoarchitecture of the deeper layers of the dorsal horn somewhat more ambiguous (Rexed, 1952; reviewed in Brown, 1981), but the physiological properties of the neurons found in these regions are equivocal as well. Multireceptive interneurons are abundant in the deeper layers of the dorsal horn (laminae III-VI) since there is convergence from several different modalities onto these cells. Mechanical, thermal, proprioceptive and visceral modalities of both noxious and innocuous origin are known to stimulate the

neurons of these laminae. Neurons of laminae III and IV generally respond to innocuous stimulation to the skin, though cells have been discovered that react to noxious inputs (Wall, 1967), and many of these cells contribute to ascending tracts that are known to convey pain information to higher centers (spinocervical and spinothalamic tracts). Neurons in lamina V also contribute to ascending tracts, and the cells in this layer and lamina VI are considered multireceptive.

The remaining layers of the spinal cord (laminae VII-X) are more grouped than they are layered. For the most part these laminae contain interneurons that connect the dorsal horn to the motorneuron pools in lamina IX. Aside from the motorneuron pools, these regions are not as well known physiologically as are the dorsal horn layers. The cells in lamina X have been associated with nociceptive inputs (reviewed in Willis, 1985), though this is not surprising since the spinothalamic tract carrying nociceptive signals to the brain crosses the spinal cord through this region.

Segmental Control of Pain

The segmental reflexes, whether monosynaptic or polysynaptic, are subject to modulation in the central nervous system. The gate control theory of Melzack and Wall (1965) suggested that interneurons may play a role in affecting sensory inputs. In this model, interneurons in the substantia gelatinosa are thought to exert presynaptic inhibition on the terminals of primary afferents. In turn, these same

interneurons are regulated by the incoming afferents: large diameter fibers excite these cells, and small fibers inhibit them. Thus, nociceptive transmission is reduced by inputs over large diameter fibers, but is enhanced by the small diameter fibers. Although physiological evidence to support this presynaptic mechanism is controversial (reviewed in Nathan, 1976; Besson and Chaouch, 1987), clinical studies at least superficially appear to agree with such a mechanism: mechanical vibration to stimulate large afferent fibers in human subjects has produced an increase in pain threshold (Ekblom and Hansson, 1982), and also relief from chronic pain (Lundberg, 1984).

Control of impulses from peripheral afferents may be exerted at the same level of entry, but may also be influenced by other segments of the spinal cord. Propriospinal neurons, which arise from and terminate entirely within the spinal cord, may serve to affect reflexes at different segments of the spinal cord, and have been indicated in the coordination of reflexes between forelimbs and hindlimbs (Jankowska et al., 1973). Propriospinal neurons are found in columns of the lateral grey horn, and may terminate on interneurons or motorneurons, resulting in excitation or inhibition of those neurons (Jankowska et al., 1974). Because propriospinal neurons are known to have connections with tracts that descend into the spinal cord from the brain, they may play a critical role in the integration of the reflexes with higher centers.

Descending Modulation of the FR

Discovery. One of the countless landmark observations on the FR provided by Sherrington and his colleagues was that of inhibitory influences on this reflex. Comparing decerebrate to spinal preparations in cats, Sherrington noted that the FR was much more excitable in the spinal state, and therefore was under "some degree of inhibition" in the decerebrate condition (Sherrington and Sowton, 1915). Expanding on this idea, Fulton (1926) proposed that spinal transection eliminated tonic inhibitory influences descending from cortical regions, possibly affecting the interneurons of the FR arc. An overwhelming amount of evidence has since been discovered to support descending suppression of spinal reflexes (e.g., Liddell et al., 1932; Kleyntjens et al., 1955; Eccles and Lundberg, 1959; Anden et al., 1964, 1966; Engberg et al., 1965, 1968; Wall, 1967; Nygren and Olson, 1977; Mokha et al., 1986; Cavallari and Pettersson, 1989).

Spinal Sites of Action. In an effort to determine where in the spinal cord pathways descend to influence the FR, Liddell et al. (1932) cut selected portions of the spinal cord and monitored the FR. They discovered that sectioning the dorsolateral and ventral funiculi increased the magnitude of FR in the decerebrate cat. Several investigators since then have confirmed that the dorsolateral funiculus is a route for descending input (Holmqvist and Lundberg, 1959; Engberg et al., 1968; McMahon and Wall, 1988), and also that some

pathways descend in the ventral columns of the spinal cord (Jankowska et al., 1968; Proudfit and Anderson, 1973).

After it was well established that the spinal cord contains pathways that descend from supraspinal centers to affect the reflexes at the segmental level, investigation began to focus on the specific neurons of the spinal cord to determine where the modulation of these reflexes might be occurring. A considerable amount of research has indicated that descending control is exerted at interneuronal elements of polysynaptic reflex arcs (Fulton, 1926; Wall, 1967; Engberg et al., 1968; Cervero et al., 1979; Cavallari and Pettersson, 1989). Wall (1967) reduced conduction through the spinal cord at the thoracolumbar junction by using a cold block to reduce the temperature, and discovered that neurons in laminae IV, V and VI were more excitable. In further studies, multireceptive dorsal horn cells that respond to noxious stimuli (including electrically activated unmyelinated fibers) were found to be inhibited by descending paths, but cells responding to non-nociceptive inputs (from large, myelinated fibers) were less affected by descending paths in decerebrate cats (Brown, 1971) and anesthetized cats (Duggan et al., 1977). Holmqvist and Lundberg (1959) determined that descending pathways exert inhibitory control on reflexes evoked from muscle and tendon (groups Ib, II and III) but not on paths excited by Ia afferents. This finding is of interest because the neural arc involving Ia muscle spindle afferents

is a monosynaptic pathway. This evidence might lead one to believe, then, that regulatory descending pathways are not effective at the motorneuron of the reflex arc. However, several investigators have measured both inhibitory and excitatory changes in motorneurons due to descending influences, and these pathways descend in the ventral white matter of the spinal cord (Jankowska, 1968; Clineschmidt and Anderson, 1970; Proudfit and Anderson, 1973).

Supraspinal Origins of Descending Pathways and Their Effects Upon the Spinal Cord. Several different sites in the brain and brainstem have been identified as sources of neurons that project to the spinal cord to exert control over reflexes. These sites include the cortex, hypothalamus, midbrain, pons, medulla, and also the cerebellum (reviews in Willis, 1982; Besson and Chaouch, 1987; Patton, 1989). These "supraspinal" centers were initially discovered by electrically stimulating discrete brain regions while examining the FR, though more advanced techniques such as axon labeling with dyes and immunocytochemistry have allowed a more precise identification of the regions responsible for control. Some of these regions produce excitatory effects in spinal cord neurons, though the net influence of these centers appears to be inhibitory. Although the detailed effects of descending paths on the FR are contradictory, some generalizations can yet be made.

A direct connection from the cerebral cortex to the spinal cord, the **corticospinal tract**, originates in the pre- and post-central gyri of the cortex (sensorimotor cortex), and travels through the midbrain as the pyramidal tract. After crossing over at the spino-medullary junction, fibers then descend in the dorsolateral white columns of the spinal cord (in most species) and enter the grey matter to terminate primarily in laminae V - VIII; therefore, this pathway directly affects interneurons, and produces both excitatory and inhibitory post-synaptic potentials (EPSPs and IPSPs, respectively) in these spinal neurons. The inhibitory actions, however, are thought to be a result of the corticospinal stimulation of interneurons that convey inhibitory impulses to the cells from which IPSPs were recorded (Lundberg, 1966). The general effect of corticospinal pathways, then, is facilitatory on polysynaptic pathways evoked from nearly all afferents. Although direct contacts with motorneurons are not thought to exist in the cat, they have been discovered in primates and humans (Kuypers et al., 1964). The net effects of corticospinal pathways are excitatory on flexor motorneurons and inhibitory on extensor motorneurons; again, these effects may be due to indirect circuits through interneurons (Lundberg, 1966).

The **rubrospinal tract** is another brain center known to influence spinal reflexes. Originating from the red nucleus in the midbrain, the axons from this nucleus descend in the

dorsolateral funiculus near the corticospinal tract. The fibers terminate in the lateral grey intermediate zone (laminae V - VII), and, like the corticospinal tract, the modulation is thought to occur at the interneuronal level. Though it has been determined that fibers of this tract do not synapse directly with motorneurons, both EPSPs and IPSPs have been recorded from motorneurons after stimulation in the red nucleus (Hongo et al., 1969). Extensive research with the rubrospinal tract by Hongo et al. (1969a,b; 1972) revealed that reflex arcs from many afferents are facilitated. However, they did indicate that reflexes initiated by FRA (which includes noxious inputs) were usually not excited and were at times inhibited by stimulation in the red nucleus.

The **vestibulospinal** tract is a well-characterized pathway from the brain to the spinal cord (reviewed in Brodal, 1969). Arising from the vestibular nuclei are actually two different paths: the lateral vestibulospinal and medial vestibulospinal tracts. The former tract descends in the ventral funiculus of the spinal cord to lumbosacral levels, where it terminates in laminae VII and VIII, and may enter the motorneuron pools. This tract is known to excite extensor motorneurons and to inhibit flexor motorneurons, and is believed to play a significant role in establishing "decerebrate rigidity" upon removal of the cerebrum. This tract does not have a marked effect upon reflex arcs evoked by FRA. The medial vestibulospinal tract also descends in the ventral columns,

but only to the level of the thoracic cord. Receiving information from the vestibular apparatus, this path is believed to mediate muscle tone in axial muscles of the upper body in relation to head movements.

The **reticulospinal** tract is a route for several pathways descending to the spinal cord to inhibit reflexes. The reticular formation in the brainstem spans from the mesencephalon to the caudalmost medulla, and contains well-defined nuclei, especially in the pons and medulla. Nuclei from the area of the pons project axons down the ipsilateral ventral funiculus, and from the area of the medulla project down the lateral funiculus. The terminations of these tracts are found predominantly in laminae VII and VIII, and probably influence reflexes by way of interneurons. On the other hand, the ventral funiculi may project to lamina IX, and IPSPs have been recorded in motorneurons after stimulation in the reticular formation (Llinas and Terzuolo, 1964).

The primary nucleus of the medullary reticular formation, the nucleus raphe magnus (NRM), has been given considerable attention, especially by Lundberg and associates (Holmqvist and Lundberg, 1961; Engberg et al., 1968). Stimulation in this brainstem area results in pronounced inhibition of reflex pathways evoked by FRAs. Not only is it inhibitory on the FR pathway, but stimulation in the NRM has demonstrated a preferential inhibition of small diameter (A-delta and C

fiber) afferent inputs over larger myelinated fibers (Willis, et al., 1977; Mokha, et al., 1985).

Another primary region of the reticular formation is found in the pons. The nucleus locus coeruleus (LC) has also received considerable attention recently (Hodge et al., 1983; Mokha et al., 1985, 1986; Buchanan and Nornes, 1986; Fung and Barnes, 1987). This nucleus contains the major aggregation of noradrenaline cells in the central nervous system (Dahlstrom and Fuxe, 1964), and fibers from this region terminate in the ventral horn, where noradrenergic terminals are abundant (Westlund and Coulter, 1980). Stimulation in the LC has been shown to strongly inhibit multireceptive interneurons recorded from laminae III to VI (Mokha et al., 1985, 1986).

A different region of the brainstem known to provide inhibitory input to spinal reflexes is the periaqueductal gray (PAG) in the midbrain. Projections from this site to the spinal cord are not well understood, but the PAG may exert its effects in conjunction with the NRM, since fibers from the PAG project to the NRM (Besson and Chaouch, 1987). Stimulation in this brainstem region has been known to provide behavioral analgesia in animals during surgery (Reynolds, 1969). Several studies have indicated that stimulation in this site results in an inhibition of the nociceptive responses of interneurons, but not the responses to innocuous stimuli of those cells (Guilbaud et al., 1977; Carstens et al., 1979, 1980).

Overview Summary

Several descending pathways are known to mediate reflexes at the spinal level. Though some effects are excitatory to neurons in the spinal cord, inhibition appears to be the net effect in the regulation of spinal reflex pathways.

Decerebrate preparations obviously lack input from corticospinal tracts, which would provide excitatory input to reflex pathways. Although rubrospinal pathways have been shown to be facilitatory upon interneurons, this control system is inhibitory to reflex pathways evoked by FRAs. The vestibulospinal path has little effect upon transmission through the FR arc, and inhibits flexor motorneurons. The reticulospinal system and neighboring brainstem sites not only produce analgesia upon stimulation but are known to profoundly inhibit reflex pathways evoked by noxious stimuli (Mokha et al., 1985). Most interesting is the discovery that the inhibitory effects by some of these descending routes seem to be applied most predominantly on the FR pathways associated with noxious inputs, indicating that C fiber contributions are affected more than are large fiber inputs (Guilbaud et al., 1977; Rivot et al., 1980; Gebhart et al., 1983). Considering those results, it might logically follow that the long-latency component of the FR, which is evoked by C fibers, would be under a relatively greater degree of inhibition in the decerebrate condition. With this in mind, the following

project was undertaken to examine the effects of spinal transection on the early and late components of the FR.

Transection of the spinal cord abolishes descending inputs that inhibit the FR; therefore, we expected to see an enhancement of the FR by cutting the spinal cord. Furthermore, since the long latency component of this reflex is believed to be inhibited more than the short latency component, we hypothesized that the late component of the FR should be enhanced more than the early component after spinal transection. In addition, knowing that propriospinal neurons are involved with the integration of sensory information between different levels of the spinal cord, we wanted to determine if cutting the spinal cord at different levels revealed any significant changes in the response of the FR.

METHODS

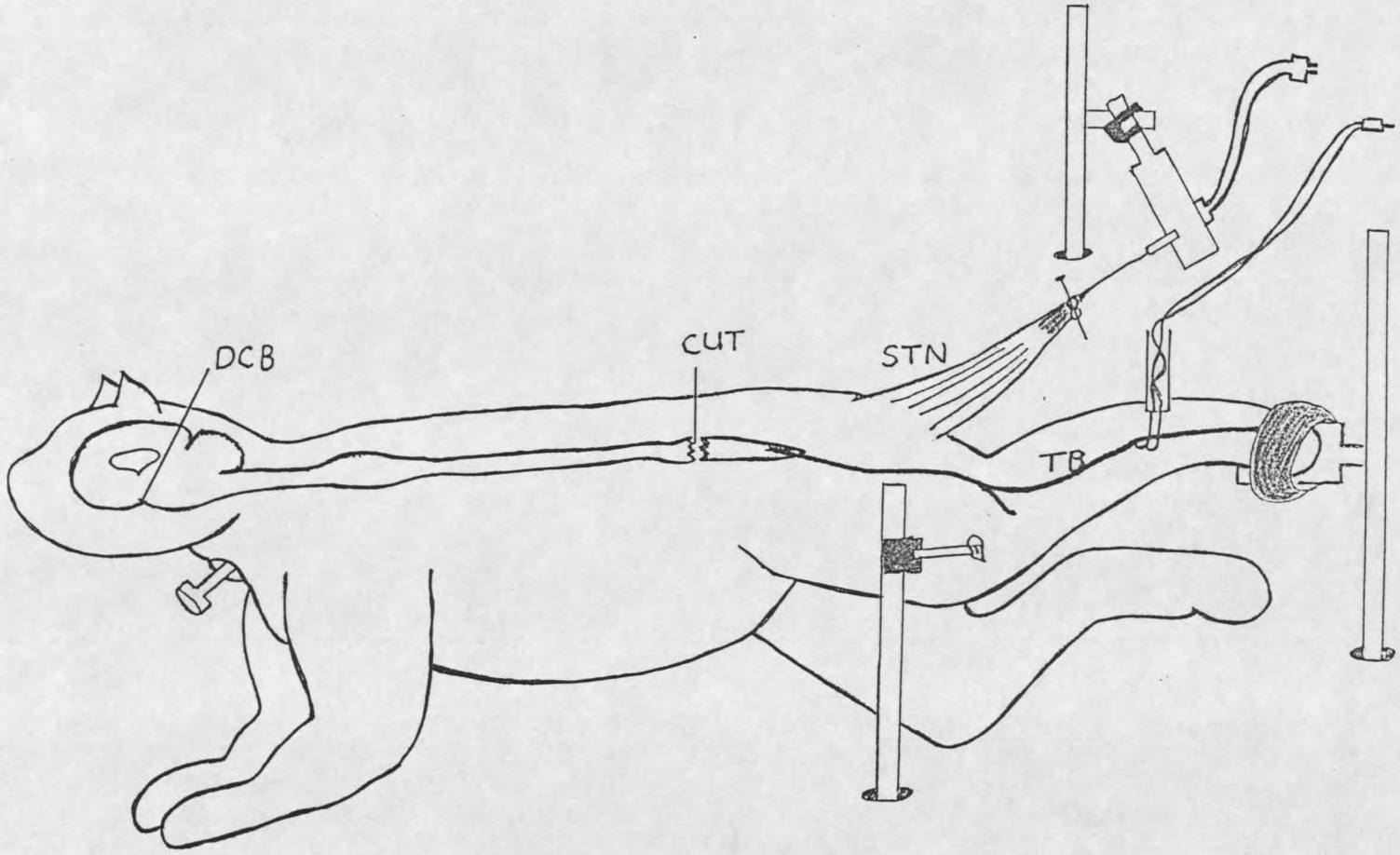
Surgical Preparation

Both male and female adult cats, ranging in weight from 1.2 to 5.0 kilograms, were used in this study. The animals were initially anesthetized intraperitoneally with Ketamine HCl, at a dose of 50 mg/kg. Additional anesthetic was administered intravenously when necessary to keep an animal at a proper level of analgesia throughout the surgical procedures. An adequate depth of anesthesia was monitored by observing the withdrawal reflex evoked by pinching the skin of a limb, and also by the palpebral reflex evoked by touching a cotton swab to the medial palpebral fold.

The external jugular vein was cannulated in order to administer saline and additional anesthetic when needed. The common carotid arteries were ligated in order to minimize blood flow to the forebrain, but the vertebral arteries which supply the brainstem were left intact. A tracheal cannula was inserted for purposes of artificial ventilation if necessary. End tidal CO_2 was monitored on each animal and maintained in the range of 3-5%.

Figure 2 illustrates the experimental design explained below. The animal's head was fixed into a stereotaxic frame. The cerebrum was removed by performing a mid-collicular decerebration just anterior to the tentorium,

Figure 2. Experimental design. The spinal frame and stereotaxic frame are not included. Hook electrodes are shown stimulating the tibial nerve (TB). The semitendinosus muscle (STN) is attached to a force transducer, which can relay to polygraph and computer. The approximate sites of decerebration (DCB) and spinal transection (CUT), respectively, are also indicated.



using a blunt spatula to separate and remove the brain tissue. To reduce blood flow into the cranial cavity during this procedure the vertebral arteries were manually occluded against the transverse processes of the atlas vertebra.

The tibial nerve was dissected free near the calcaneal tendon of the left hindlimb, ligated as far distad as possible, then severed. Not only does this allow for easy handling of the nerve, but it also eliminates conduction to or from the foot. The nerve was then exposed in the popliteal fossa where it branches from the sciatic nerve. The peroneal branch of the sciatic nerve was ligated and severed to eliminate extraneous activity in the limb.

Either nichrome hook or stainless steel indwelling electrodes were used for stimulation. When using the hook electrodes, stopcock grease was applied around the nerve and electrodes, and a small square of parafilm covered this arrangement to isolate it from nearby tissues. When using the indwelling electrodes, both electrodes and nerve were threaded through a 2 cm piece of polyethylene tube, and stopcock grease filled the tube to insulate and secure the array.

The semitendinosus muscle was dissected free from its site of insertion on the tibia. A ligature was tied to the tendon of insertion, and to prevent it from slipping off, the ligature was secured to an insect pin passed through the tendon (see Fig 2). A free end of the ligature was then used to attach the semitendinosus to a force transducer, which

converts mechanical energy of the muscle to an electrical signal.

The cat was then placed in a spinal frame apparatus, suspending the body from the spinous process of a cervical and a lumbar vertebra, respectively. The left hindlimb was stabilized by inserting a drill bit through the distal end of the femur and clamping the bit to an upright rod fastened to the table. The foot of the left hindlimb was also fixed to an upright rod with plaster cast material.

With the animal secure, the spinal cord was exposed by laminectomy near the T12-L1 (and in 6 cats also the upper cervical) spinal segments so that it could be transected after data collection in the decerebrate state was complete. Transection of the spinal cord was performed with a battery-operated cautery unit: this helped to reduce bleeding around the site. To prevent desiccation and to keep the spinal cord near normal temperature, warm mineral oil was poured around the exposed spinal cord. After data were collected, the animal was euthanatized with an overdose of sodium pentobarbital.

Stimulation and Recording

Stimuli consisted of square wave pulses (1 ms) delivered from a waveform generator through a constant current isolation unit. Isometric tension produced by the semitendinosus muscle during the reflex was measured with a force transducer, the

output of which was fed into a Beckman R612 polygraph and also into a computer-based data acquisition program (RC Electronics).

Four indices of excitability were evaluated, first in the decerebrate and then in the spinal state. (In the case of those animals cut both at the cervical and thoracic areas of the cord, data were collected after each cut.) The first of these indices was the **threshold current** required to evoke a response of the muscle at low frequency (1 Hz) and high frequency (10 Hz) stimulation to the nerve. The second was the **latency** of the reflex response, i.e., the delay from the time of stimulus to the first noticeable response. The third index evaluated was the **time to peak tension** while stimulating at a level high enough to evoke a maximal response (usually 1 mA or more). The fourth index was that of **time to decay** to 50% of the peak tension, which indicates the duration of the response as well as the "late" component of the reflex.

RESULTS

General Observations

Data were collected from twenty five cats, although in some animals we were unable to obtain all measurements. Some general characteristics were noted for each of the animals in our preparation. In most cats increased extensor muscle tone was observed immediately after decerebration. All four limbs responded by stretching outward from their relaxed positions. Even in those animals where the extensor rigidity was not as dramatic after decerebration, extensor tone could be demonstrated by passively flexing a limb. Due to an increase in the excitability of the stretch reflex, the extensor muscles forcibly opposed any passive flexion we applied to a limb. In addition to the extensor tone, vigorous responses of the patellar tendon knee jerk reflex were evoked by tapping the tendon just distad to the patella.

The physiological condition of the cats remained fairly stable after surgical preparation. Indeed, few animals required artificial ventilation immediately after decerebration, and some maintained well on their own after the muscle and nerve were prepared. Some animals required no intervention even after transection of the spinal cord and up until the end of the experiment. However, the ability of a cat to maintain breathing on its own was not requisite for

evoking good reflex responses. Many of the animals that were artificially ventilated showed active responses to our stimulation.

Severing the spinal cord is obviously an overwhelming stimulus to an animal, and our cats, although fairly secure in a stereotaxic and spinal frame, demonstrated marked responses. Typically the animal's back arched up as far as the spinal frame would allow, and the respiration rate increased slightly. Within a minute or less breathing was normally stabilized and the animal's body relaxed.

In the forelimbs of two animals a "walking" sort of motion was observed after decerebration, where each forelimb would alternately extend then retract in rhythmic movement. This motion sometimes continued for several minutes at a time: in one animal it continued throughout the experiment.

Distribution of Thresholds

Of the four main indices of excitability, the threshold required to evoke the FR was the first to be examined, and those data will be presented first and separate from the other three indices (latency, time to peak tension and time to decay) evaluated for each animal. Threshold measurement is one way to determine the excitability of the FR in an animal, especially as it relates to the class of afferent fibers that must be activated to evoke the reflex.

A comparison of thresholds was made for six animals whose spinal cords were cut at both the cervical and thoracic levels, and those data are presented at the end of this section. To avoid any confusion, the term "spinal" when used hereafter refers to the **thoracic** spinal condition, unless otherwise noted.

Complete measurements of all four threshold parameters (decerebrate at 1 Hz and 10 Hz, spinal at 1 Hz and 10 Hz) were made in eighteen of the twenty five animals. A considerably wide range of threshold values was obtained in all conditions: in the decerebrate animals stimulated at 1 Hz the distribution of these values was the most irregular.

Decerebrate at 1 Hz

The most variable data were obtained from twenty two decerebrate cats stimulated at 1 Hz (Fig 3). The thresholds in this state were typically higher than in any other, and often it was difficult to obtain a single response in the decerebrate condition until stimulation at high intensities (1 mA or more). Values ranged from a low of 0.035 mA to a high of 5.00 mA, with an average of 1.53 mA. A standard error of measurement (SEM) was calculated to be 0.38.

Decerebrate at 10 Hz

Thresholds for decerebrate animals stimulated at 10 Hz were consistently much lower than those recorded at 1 Hz in this state, which would be expected for the FR at a higher

frequency because of temporal summation. The range of thresholds in twenty three cats was from 0.04 to 5.00 mA; however, values above 0.50 mA were discovered in only three cases (Fig 4). The average value for this condition was 0.66 mA, and SEM = 0.30.

Spinal at 1 Hz

After transecting the spinal cord, the threshold required to evoke the FR at 1 Hz was typically much lower than in the decerebrate state. The average value for this condition was 0.39 mA, well below the average found at 1 Hz in the decerebrate condition. The SEM was 0.18. In only three of twenty four cats were thresholds found above 0.50 mA (Fig 5). The values ranged from a low of 0.014 mA to a high of 4.00 mA. In three animals where no reflex could be evoked at any intensity in the decerebrate preparation at 1 Hz, reflexes with thresholds of 0.85, 0.50, and 0.08 mA, respectively, were seen in these animals after cutting the spinal cord.

Spinal at 10 Hz

The lowest thresholds were commonly observed in spinal animals when stimulating at 10 Hz. The range of values in this preparation was from 0.02 to 2.00 mA, but in only two of twenty one animals were the thresholds observed above 0.25 mA (Fig 6). The average value for this condition was calculated at 0.21 mA, with a SEM = 0.10

Figure 3. Distribution of thresholds at 1 Hz in the decerebrate preparation. Average value is 1.53 mA.

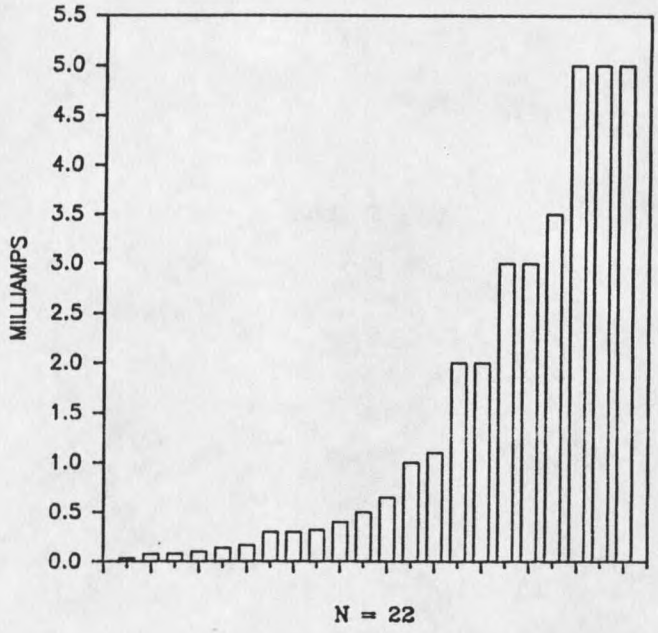


Figure 4. Distribution of thresholds at 10 Hz in the decerebrate preparation. Scale change is indicated for the last three values. Average value is 0.66 mA.

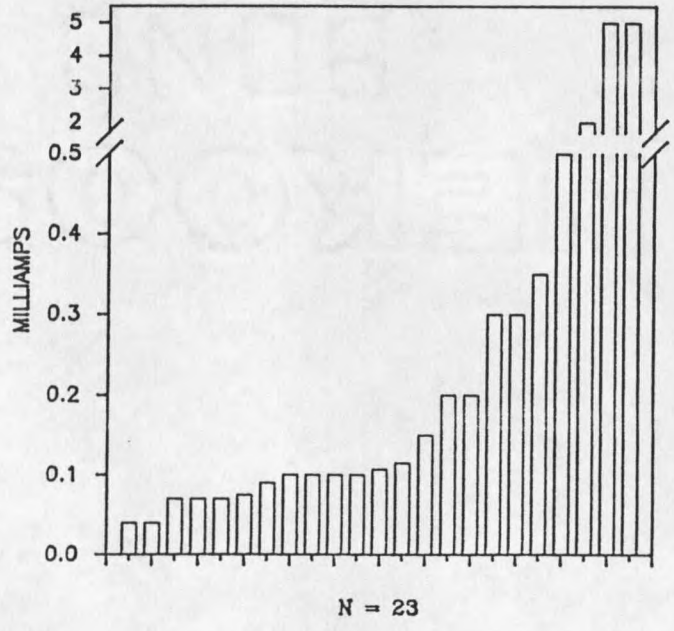


Figure 5. Distribution of thresholds at 1 Hz in the spinal preparation. Scale change is indicated for the last three values. Average value is 0.39 mA.

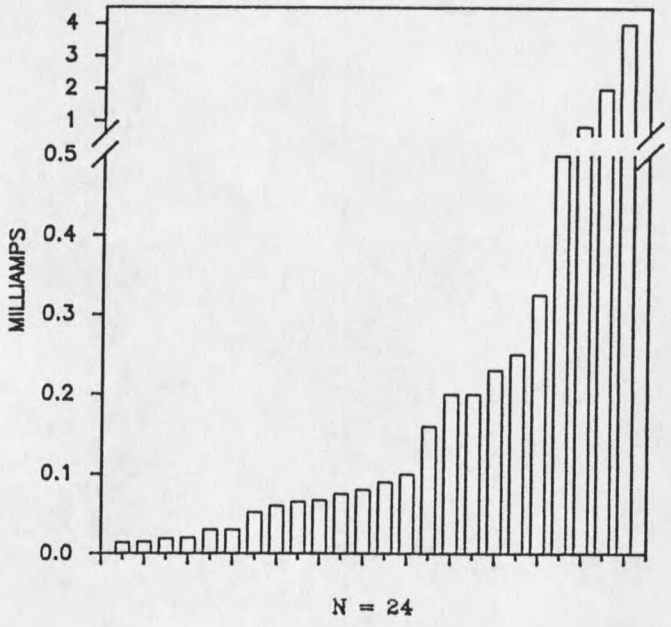
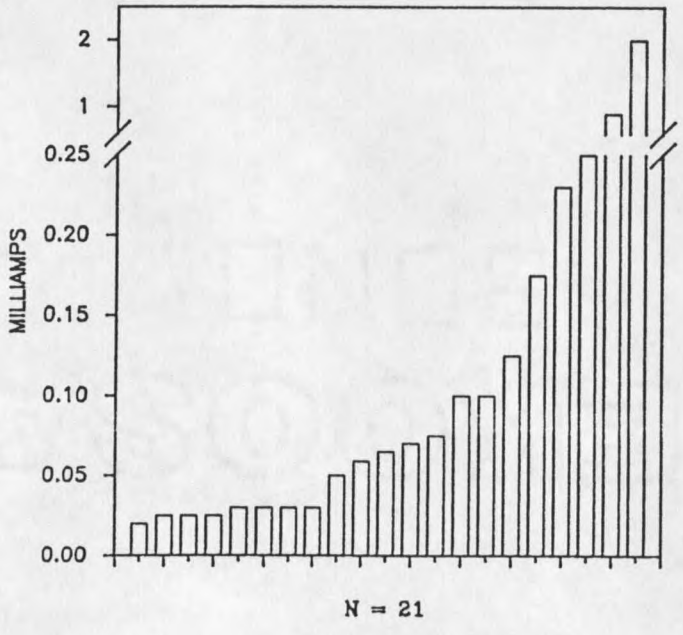


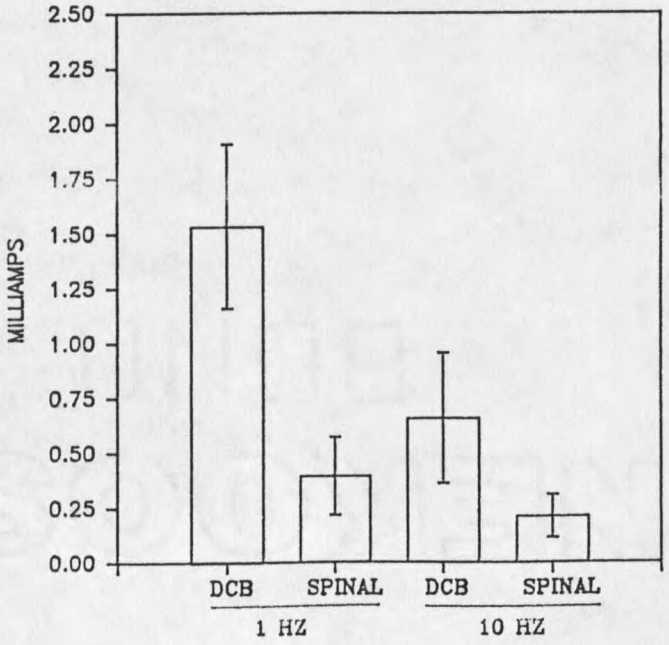
Figure 6. Distribution of thresholds at 10 Hz in the spinal preparation. Scale change is indicated for the last two values. Average value is 0.21 mA.



Comparison of Thresholds

Figure 7 summarizes the average thresholds measured at 1 Hz and 10 Hz in both the decerebrate and spinal conditions. Immediately upon spinal transection in most animals an increased responsiveness to stimuli was witnessed. However, after spinal cord transection in three cats there was an increase in the threshold at 1 Hz, and these three plus two more showed an increase in the threshold at 10 Hz. (Only one of these five cats showed a conspicuous increase: from 0.17 to 2.00 mA at 1 Hz, and from 0.115 to 2.00 mA at 10 Hz.)

Figure 7. Average threshold values measured at both 1 Hz and 10 Hz in the decerebrate and spinal conditions. Histograms include SEM (see text for values).



A t-paired test was used to analyze the differences between the various distributions. At 1 Hz the difference in thresholds between decerebrate and spinal preparations was statistically significant ($p = .004$), while at 10 Hz it was not ($p = .17$). The average threshold at 1 Hz in the decerebrate condition was also significantly different from both decerebrate and spinal thresholds at 10 Hz ($p < .015$ and .005, respectively).

Decerebrate/Spinal Ratios

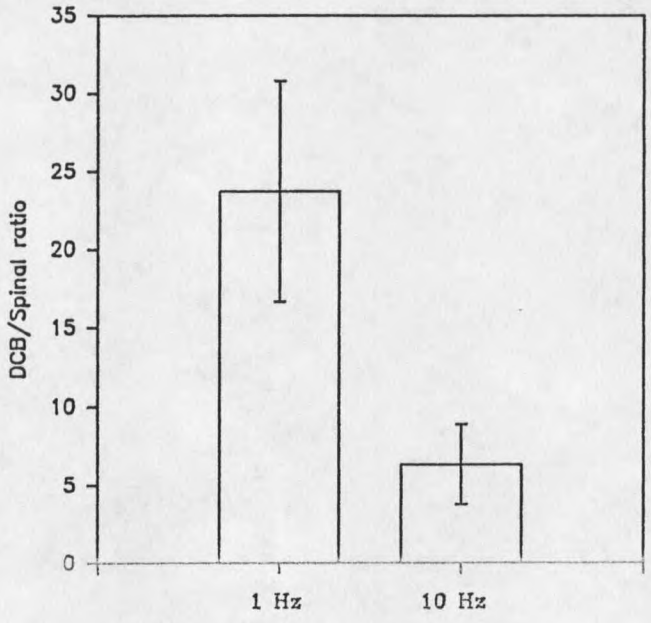
To further evaluate the difference in excitability between the decerebrate and spinal conditions, a decerebrate/spinal threshold ratio was calculated for each cat. Figure 8 shows the average of these ratios out of 21 cats at both 1 Hz and 10 Hz. The average ratio at 1 Hz was 23.7, while at 10 Hz the average was 6.3. A t-single test was used to evaluate the null hypothesis that the ratio would be 1.0 (i.e. decerebrate and spinal values would be equal). The difference in ratios for 1 Hz was highly significant ($p < .005$), while the ratio at 10 Hz was just significantly different ($p = .05$).

Cervical Spinal vs.Thoracic Spinal

Table 2 shows the comparison of threshold values recorded when the spinal cord was transected at the cervical level versus the thoracic level in six cats. The data obtained in

the decerebrate condition are included for comparison. In five of the six animals there was no considerable difference in threshold values between the two preparations; therefore, thoracic spinal data from these animals were considered with the data from all other animals prepared with a thoracic spinal transection.

Figure 8. Average decerebrate/spinal threshold ratios measured at 1 Hz and 10 Hz. Histograms include SEM (see text for values).



Analysis of Muscle Force Recordings

In addition to the changes in threshold, the excitability of the FR was examined by evaluating three attributes of the response: (i) the **latency** between stimulus and response, (ii) the **time to rise** to peak tension, and (iii) the **time to decay**

to 50% of the peak tension. Responses of the FR were examined on the computer, although a trace of the reflex activity was not always saved. With some animals it was difficult to obtain a reading that was suitable for measure, either because it was not strong enough to produce a good trace or because successive waveforms were not consistent enough (even though threshold remained the same). The three indices were measured in the decerebrate (n=10) and spinal (n=18) conditions, and then a decerebrate/spinal ratio was calculated for each (Table 3).

Summary of Time Measurement

Latency. The time delay between stimulus and response is the latency. Although it would be more accurate to record this time delay from motorneurons, our measurement of the first noticeable muscle response after stimulation does provide a method of quantifying the time required for the FR to ensue. The average latencies measured in the decerebrate and spinal conditions are listed in Table 3. An average ratio of decerebrate/spinal latencies was calculated at 2.2, indicating that in the spinal state, the response of the muscle (more precisely the recruitment of motorneurons) was about twice as fast as that in the decerebrate state. Figure 9 illustrates an example of the difference in latency between decerebrate and spinal preparations.

Since the time values in the spinal condition were consistently lower than those in the decerebrate condition, a

one-tailed t-single test was used to evaluate the differences between these values. Against a hypothesized decerebrate/spinal ratio of 1, these values differ significantly (p= .05).

Table 2. Differential effects of cervical vs. thoracic spinal cord transection on FR thresholds. All values are in milliamps.

Case No.	1 Hz			10 Hz		
	DCB	SPINAL		DCB	SPINAL	
		C-2	T-12		C-2	T-12
1	.035	.070	.060	.070	.085	.070
2	.080	4.500	.325	.070	.350	.300
3	5.000	1.000	4.000	.070	.040	.030
4	.100	.040	.020	.040	.100	.030
5	2.000	.030	.019	.090	.040	.040
6	.400	.100	.030	.100	.015	.030

Table 3. Analysis of single reflex responses, decerebrate vs. spinal. All values are in milliseconds.

MEASUREMENT	DCB (n=10)	SPINAL (n=18)	AVERAGE DCB/SPINAL RATIO (n=8)
Latency	26.729	17.472	2.169
Rise	197.518	85.194	2.516
Decay	392.375	183.110	2.067

Rise Time. The time required for the response to reach peak amplitude, instead of the peak amplitude itself (which is

often measured to evaluate the FR response) was chosen as another index of reflex excitability. An average decerebrate/spinal ratio of 2.5 (Table 3) revealed that in the spinal condition the time required to reach peak tension was reduced to less than half that of the decerebrate condition. Figure 10 illustrates the effects of spinal transection on rise time and also decay time (discussed next).

Values in the spinal state were always lower than the decerebrate state for rise time, thus their difference was evaluated similar to the latency index, and determined to be highly significant ($p < .01$).

Decay Time. The final index of FR excitability measured was that of the time required for the reflex response to decrease to 50% of the peak amplitude, or the decay time. Using this index we can evaluate the duration of the FR, and also the effects upon the late component of the reflex. The average decerebrate/spinal ratio of 2.1 (Table 3) indicates that the decay time was reduced by approximately one half in the spinal state compared to the decerebrate state; in other words, the discharge of motorneurons lasted only half as long in the spinal state. Decay time was reduced in all cats, therefore statistics were performed as described for latency and rise times. The difference of decay time between decerebrate and spinal preparations was very significant ($p = .01$)

The decreased latency and rise times, and more rapid decay of the individual muscle responses in the spinal state were quite consistent. In just one animal there was an increase in the latency time in the spinal state compared to the decerebrate state, and none of the cats revealed either an increase in the rise time or prolonged decay of the FR after spinal transection. Table 4 is provided for a comprehensive summary of data collected on all animals.

Figure 9. Effect of spinal transection on the latency of the FR. Both records are averages of ten responses evoked by stimulating at 1 Hz, 0.2 mA. In the decerebrate condition (trace A), the latency was 65 msec. After cutting the spinal cord of this animal (trace B), the latency measured only 18 msec.

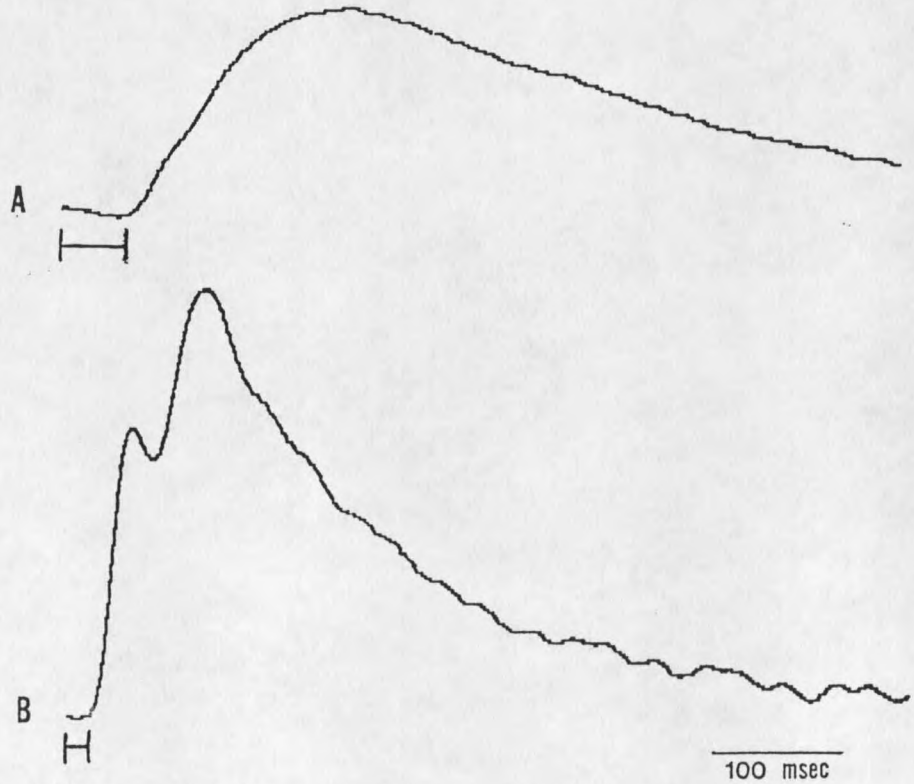


Figure 10. Effect of spinal transection on the rise time and decay time of the FR. The reflex was evoked by stimulating at 0.5 Hz and 4.0 mA. In the decerebrate state (trace A) the rise and decay times were 474 msec and 962 msec, respectively. In the spinal state (trace B) the values decreased to 81 and 237 msec, respectively.

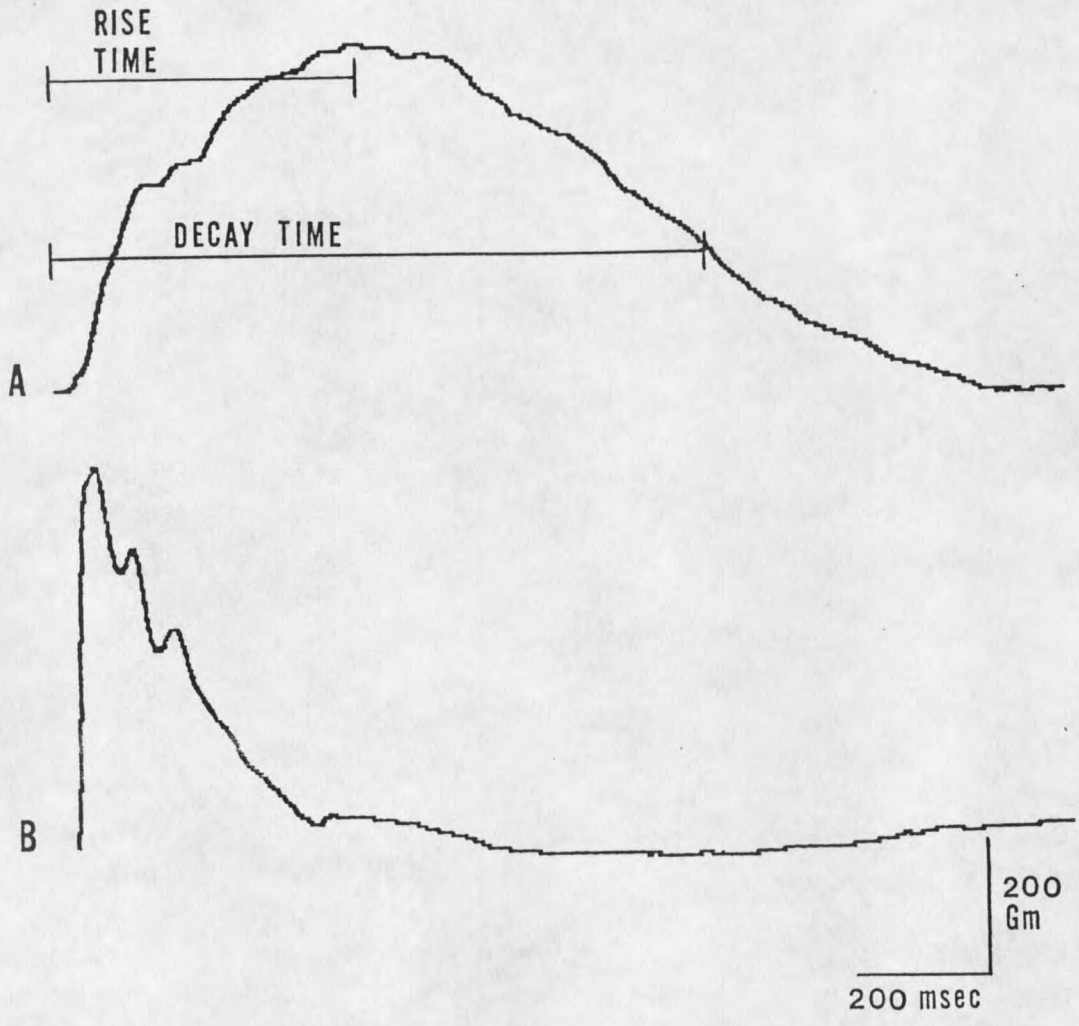


Table 4. Summary of indices of FR excitability recorded from all animals. Threshold values are listed in the first four columns after each animal (units = mA), and temporal indices follow in the last three columns as decerebrate/spinal ratios (units = mS). Values not recorded are indicated by dashes. DCB = decerebrate, Late. = latency.

Cat	DCB		Spinal		Late.	Rise	Decay
	1 Hz	10 Hz	1 Hz	10 Hz			
1	0.650	-----	0.090	-----	19.8/ 15.0	257.7/ 109.2	355.3/ 206.4
2	0.030	-----	0.250	-----	-----	-----	-----
3	0.500	0.300	0.200	0.250	----/ 21.6	----/ 105.4	----/ 177.3
4	0.035	0.070	0.060	0.070	11.7/ 17.9	105.7/ 69.2	195.9/ 164.4
5	0.080	0.070	0.325	0.300	17.8/ 17.0	146.8/ 92.7	332.2/ 162.2
6	0.077	0.040	-----	-----	11.7/ ----	117.4/ ----	321.3/ ----
7	5.000	5.000	0.100	0.100	----/ 18.5	----/ 93.9	----/ 160.7
8	3.500	5.000	0.200	0.175	24.7/ 15.5	135.9/ 92.7	291.7/ 193.2
9	0.140	0.100	0.015	0.125	55.6/ 11.1	222.5/ 111.2	474.6/ 210.1
10	3.000	0.100	0.030	0.025	----/ 19.8	----/ 104.4	----/ 248.4
11	0.320	0.100	0.052	0.050	----/ 21.0	----/ 72.9	----/ 241.0
12	2.000	0.150	0.065	0.065	----/ 21.6	----/ 93.9	----/ 166.8
13	3.000	0.500	0.075	0.075	----/ 24.1	----/ 105.1	----/ 188.5
14	0.300	0.075	0.014	0.020	27.9/ 24.1	112.8/ 55.6	214.0/ 208.7

Table 4, continued

Cat	DCB		Spinal		Late.	Rise	Decay
	1 Hz	10 Hz	1 Hz	10 Hz			
15	1.000	0.200	0.230	0.230	25.8/ 15.2	471.7/ 62.2	955.5/ 200.2
16	-----	2.000	0.850	0.900	-----	-----	-----
17	-----	0.300	0.500	0.025	----/ 9.3	----/ 73.0	----/ 159.1
18	-----	0.107	0.080	0.100	-----	-----	-----
19	1.100	0.350	0.160	-----	46.4/ 7.73	262.7/ 115.9	440.4/ 231.8
20	5.000	0.200	0.067	0.059	-----	-----	-----
21	0.165	0.115	2.000	2.000	26.0/ 24.7	142.1/ 77.3	343.0/ 157.6
22	5.000	0.070	4.000	0.030	-----	-----	-----
23	0.100	0.040	0.020	0.030	----/ 15.2	----/ 39.6	----/ 83.8
24	2.000	0.090	0.019	0.025	----/ 15.1	----/ 58.9	----/ 135.9
25	0.400	0.100	0.030	0.030	-----	-----	-----

DISCUSSION

Our results confirm early reports of an increased excitability of the flexion reflex in the spinal animal compared to the decerebrate animal (Sherrington and Sowton, 1915). Single reflex responses were difficult to obtain in the decerebrate condition, which would be expected since the brainstem areas still intact in this preparation are inhibitory upon the FR. The response was evoked much more readily in the spinal state: the average threshold intensity decreased 74% after spinal transection while stimulating at 1 Hz, and decreased 68% while stimulating at 10 Hz. The reflex evoked at 1 Hz in the decerebrate state required stimulation at high intensities, i.e., it required input from the higher threshold C fibers; however, in the spinal state a maximal response was evoked from low intensity stimulation that recruited only A-delta fibers. When a stimulus strong enough to recruit C fibers was given in the spinal state, no increase was observed in the response, nor was there a change in the temporal indices measured for the reflex.

Latency, rise time and decay time were three other indices of excitability of the FR measured in this project. Both latency and rise times of the reflex response decreased by approximately one half in the spinal compared to the decerebrate preparation, and the decay of the response in the

spinal condition was nearly twice as rapid compared to the decerebrate state. Although the FR was evoked in the spinal state at intensities that recruited only A-delta fibers, no change was observed in the latency, rise or decay times of the response when C fibers were added to the volley. These data help to quantify the general observation that the FR is more excitable in the spinal state, and highlight a more important aspect of the reflex response: the duration of the FR response is significantly reduced in the spinal condition, indicating that C fibers have less of an influence on the reflex after cutting the spinal cord.

In the spinal preparation, all descending inputs from higher centers that would normally influence the FR are eliminated. These modulating pathways include the corticospinal, rubrospinal, vestibulospinal and reticulospinal. Some of these paths (particularly the corticospinal and rubrospinal) have been reported to exert excitatory influences upon interneurons and motorneurons (Appelberg et al., 1975). However, in a decerebrate animal the corticospinal tract is obviously not influential since all cerebrum is removed, nor is the rubrospinal tract considered effective in our preparation because the level of decerebration was mid-collicular, which would likely ablate most if not all of the red nucleus. Thus, at least two possible excitatory influences on spinal neurons can be omitted when considering the present results. The effect of

many supraspinal centers upon the FR are known to be inhibitory, and several areas of the brain have been given particular attention, including the PAG, NRM and LC.

The terminations of descending pathways is within the dorsal horn and intermediate zone of the spinal grey matter, where interneurons predominate; therefore, it is likely that the modulation of the FR occurs at the interneuronal level, and recordings from interneurons from various places in the spinal cord have supported this proposition (e.g., Wall, 1967; Woolf, 1983). Even so, supraspinal control at the motorneuron should not be overlooked, and possibly occurs simultaneously with effects at the interneuronal level. Simultaneous recordings from interneurons and motorneurons while stimulating several brain stem regions may be difficult, but it could provide more complete information about descending tracts and spinal reflex function.

Supraspinal axons also descend upon propriospinal neurons of the spinal cord, which serve to coordinate activity at different spinal cord levels. In six of our experiments the spinal cord was transected at the cervical as well as the thoracic level. Cutting at the cervical level would have left intact some propriospinal neurons that extend through several segments of the cord. The results of this study indicate that there was little difference in the effects on the FR between cervical and thoracic spinal transections; therefore,

propriospinal neurons from more rostral levels of the spinal cord are unlikely to be involved in modifying the FR.

Not only are the descending pathways known to be inhibitory to the FR, but they appear to exert their influences differentially upon the contributions of the different afferents that contribute to this reflex. Mokha et al. (1985) demonstrated that stimulation in the nucleus raphe magnus and locus coeruleus both produced inhibitory actions in multireceptive neurons of the dorsal horn, and this inhibition was found to be most effective on the responses to C fiber inputs. This finding is of particular importance to this study, since the long latency response of the FR is believed to be the result of nociceptive C fiber inputs (Dimitrijevic and Nathan, 1970). Thus, if descending pathways are preferentially inhibitory to the C fiber inputs to the FR, then one would expect to see a relatively greater increase in the late component of the FR after spinal transection. Our results, however, do not agree with this hypothesis. Not only did we fail to observe an increase in the late component of the FR, but we consistently observed an abatement of the late component of this reflex after severing the spinal cord.

Even if the descending axons from the brain and brainstem were to form relatively simple connections with interneurons and/or motorneurons of the spinal cord, the results of this investigation do not provide evidence for simple mechanisms of the FR. The sites of action of the descending pathways are

far from conclusive, and further complication arises from a lack of understanding the functional connectivity of the neurons within the spinal cord. It is very likely that a complex array of circuits is responsible for controlling spinal reflexes, including presynaptic inhibition of afferents, modulation of interneurons that are themselves both excitatory and inhibitory, and excitation and inhibition of motorneurons. However, there may exist rather discrete populations of interneurons that react differently to A and C fibers in the absence of descending inputs, but they have not yet been identified with single unit recording. The variety of methods used to record from and stimulate interneurons has in many ways made the task of identifying these neurons easier, yet there remains much ambiguity about the properties of these neurons.

Valuable knowledge of descending influence on the FR has been gained in the past century by examining this reflex after complete transection of the spinal cord. However, a more complete analysis of brain stem interactions and spinal reflexes can be obtained by selective lesioning of different quadrants of the spinal cord. Many investigators have performed such experiments to date (reviewed in Besson and Chaouch, 1987), and those methods are presently being conducted in this laboratory (McMillan, unpublished data). Continued single unit recording and labeling of interneurons are techniques that also must be employed if we are to

understand the specific role of the many interneurons that may be involved in the neural arc of spinal reflexes.

Experimental Variation

The measurements acquired in this study were in some conditions quite variable (see Figs 3-6). This variation could result from experimental conditions, or it could be a result of innate differences between animals, or it could result from a combination of the two.

The surgical procedures necessary to prepare an animal for this type of investigation are at best considerably invasive. The physiological condition of each animal prior to the experiment would obviously affect how well it maintained after surgery, and since most of these experiments lasted eight hours or more, this is not a minor consideration. Knowing a detailed history of each animal would be helpful in evaluation, but this is simply not feasible. Just as humans differ in their responses to noxious stimuli, the cats used in this study must be considered to have a wide range of responses to the same stimuli, both between animals and within each animal.

The experimental design was kept as constant as possible, but naturally there must be an allowance for human error in reproduction of the design. The resting length of the muscle once it was attached to the force transducer was not standardized for each animal, and this may have affected the

excitability of the FR, since excessive stretch would increase the output of Ia afferents, which in turn would increase activity in motorneurons to the muscle. Another variable in procedure was that of electrode placement. Although the tibial nerve was stimulated to provide the afferent arm of the reflex, the placement of the electrodes within this nerve differed in some experiments. At times it was necessary to place the electrodes close to the bifurcation of the sciatic nerve, which could have resulted in the stimulation of other afferents from the peroneal branch.

Although the experimental procedure used in this examination of the FR can be scrutinized for its reliability, I feel that it provides a useful means of investigating the complex circuits involved in this spinal reflex and the descending influences upon them. As with any facet of science, electrophysiological study is quite limited by the available technology, and currently it is necessary to invade and disrupt the natural configuration of neural circuits, making interpretation difficult. Although much has been learned from this type of investigation, we must search for improved methods if we desire more conclusive results.

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