A SENSORY ROLE FOR THE CRUCIATE LIGAMENTS
Regulation of joint stability via reflexes onto the γ-muscle-spindle system

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ABSTRACT

Reflex effects evoked by graded electrical stimulation of the posterior articular nerves (PAN) of the ipsi- and contralateral knee joints were investigated using both micro-electrode recordings from γ-motoneurones and recordings from single muscle muscle spindle afferents. Spindle afferent responses were also recorded using natural stimulation of different types of receptors, to elucidate if the articular reflexes onto the γ-motoneurones were potent enough to significantly alter the muscle spindle afferent activity. Stretches of the ipsilateral posterior (PCL) and anterior (ACL) cruciate ligaments, pressure on the ipsi- and contralateral knee and ankle joint capsules, and passive flexion/extension movements of the joints in the contralateral hind limb were performed. The occurrence of different sensory endings in the ACL and PCL was examined using gold chloride staining for neuronal elements. All experiments were performed on chloralose anaesthetized cats.

More than 90% of the static and dynamic γ-motoneurones were responsive to electrical stimulation of the PAN. Most γ-cells responded to low intensity electrical stimulation. Excitatory reflex effects predominated on both static and dynamic posterior biceps-semiendinosus (PBSt) γ-cells, while excitatory and inhibitory effects occurred with an about equal frequency on triceps-plantaris (GS) γ-cells. The fastest segmental route for excitatory PAN effects on hind limb γ-motoneurones seems to be di- or trisynaptic, while the path for inhibitory effects seems to be at least one synaps longer.

Physiological stimulations of ipsi- and contralateral joint capsules and of ipsilateral cruciate ligaments were all found to evoke frequent and potent changes in spindle afferent responses from the GS and PBSt muscles. It was shown that these effects were due to reflexes onto dynamic and static fusimotor neurones caused by physiological activation of articular sensory endings. Both ipsi- and contralateral joint receptor stimulation evoked excitatory as well as inhibitory fusimotor effects. The highest responsiveness was found during stimulation of the cruciate ligaments, i.e. 58% for GS and 47% for PBSt primary spindle afferents to PCL stimulation, and 73% for GS and 55% for PBSt primary spindle afferents to ACL stimulation. Significant alterations in spindle afferent activity was encountered at very low traction forces applied to the cruciate ligaments (5-10 N). The low thresholds, the tonic character of the stimuli, and the fact that different types of sensory endings were demonstrated in the cruciate ligaments (i.e. Ruffini endings, Pacinian corpuscles, Golgi tendon organ like endings and free nerve endings), indicate that the fusimotor effects observed were caused by activation of slowly adapting mechanoreceptors, most likely Ruffini endings and/or Golgi tendon organ like endings.

The potent reflex effects on the muscle spindle afferents elicited by increased tension in the cruciate ligaments indicate that these ligaments may play a more important sensory role that hitherto believed, and it is suggested that they may be important in the regulation of the stiffness of muscles around the knee joint, and thereby for the joint stability. The possible clinical relevance and the mechanisms by which joint receptor afferents, via adjustment of the muscle stiffness, may control joint stability are discussed.

Key words: Joint afferent - Mechanoreceptor - Cruciate ligament - Fusimotor neuron - Reflex - Muscle spindle afferent - Motor control - Cat
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The potent reflex effects on the muscle spindle afferents elicited by increased tension in the cruciate ligaments indicate that these ligaments may play a more important sensory role that hitherto believed, and it is suggested that they may be important in the regulation of the stiffness of muscles around the knee joint, and thereby for the joint stability. The possible clinical relevance and the mechanisms by which joint receptor afferents, via adjustment of the muscle stiffness, may control joint stability are discussed.

Key words: Joint afferent - Mechanoreceptor - Cruciate ligament - Fusimotor neuron - Reflex - Muscle spindle afferent - Motor control - Cat
Writing a book is an adventure: it begins as an amusement, then it becomes a mistress, then a master, and finally a tyrant.

Sir Winston Churchill

I was working on the proof of one of my poems all the morning, and took out a comma. In the afternoon I put it back again.

Oscar Wilde
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### ACKNOWLEDGEMENTS

### REFERENCES
This thesis is based on the following papers, which in the text are referred to by their Roman numerals.


ABBREVIATIONS

The following abbreviations are used in the text:

ABSm ............... anterior biceps and semimembranosus
ACL .................. anterior cruciate ligament
CNS .................. central nervous system
D ..................... dynamic
DLF ................. dorsolateral funiculus of the spinal cord
DP ................... deep peroneal nerve
DR ................... dorsal root
EMG ................ electromyography
GS ................... gastrocnemius, soleus and/or plantaris
L ..................... lumbar
LAN ................ lateral articular nerve of the knee joint
MAN ................ medial articular nerve of the knee joint
MesADC ............ mesencephalic area for dynamic control
NR ................... nucleus ruber
PAN ................ posterior articular nerve of the knee joint
PBSt ............... posterior biceps and semitendinosus
PCL ................ posterior cruciate ligament
PM ................... cerebellar paramedian lobule
S ..................... static or sacral
T ..................... threshold
Th ................... thoracic
Tib ................... tibial nerve
INTRODUCTION

The function of the sensory information conveyed by muscle, skin and joint nerves has been a subject of great interest for many years among neurophysiologists (for reviews, see e.g. Goodwin et al. 1972; Goodwin 1976; McCloskey 1978; Matthews 1982; Proske et al. 1988). At the beginning of the century, when sophisticated muscular sensory endings like the muscle spindle and the Golgi tendon organ were described (Golgi 1903; Sherrington 1894), it became generally believed that the muscular receptors were the most important in proprioception as well as in segmental and inter-segmental regulation of limb muscle tone (see e.g. Sherrington 1906; Creed et al. 1932).

This view was further strengthened during the 1960's and 1970's due to the observations that muscle vibration (i.e. activation of muscle spindle afferents) could induce illusions of movement in stationary limbs (Hagbarth & Eklund 1966; Goodwin et al. 1972), and to the demonstrations of central projections of muscle receptor afferents (Andersson et al. 1966; Landgren & Silfvenius 1969, 1971; Lundberg & Oscarsson 1960; Oscarsson & Rosén 1963; Phillips et al. 1971; Johansson & Silfvenius 1977a,b,c). A widely accepted opinion has been, and may still be, that sufficiently strong evidence exists for a significant involvement of muscle afferents in proprioception and in normal reflex regulation of muscle activity, leaving joint and cutaneous receptor afferents to functions such as facilitatory activity and/or mediation of protective reflexes (e.g. Matthews 1972, 1977; Goodwin 1976; McCloskey 1978; Burgess et al. 1982; Clark et al. 1985; Wei et al. 1984, 1986).

However, the view that only the muscle afferents convey information relevant for proprioception and coordination has been questioned by several authors who have claimed that the role of joint receptor afferents has been oversimplified and underestimated (see e.g. Paper I-VII; Ferrell et al. 1985; Proske et al. 1988). In fact, several electrophysiologi-
cal and morphological investigations have shown that sensory endings in the joints have properties suitable for providing the CNS with information relating to limb position and movement (for references, see below: Sensory endings in the knee joint). Furthermore, it has repeatedly been demonstrated that ascending pathways transmitting messages from joint afferents project to supraspinal centres including the cerebral cortex (for references, see below: Projections of knee joint afferent activity to supraspinal structures; see also e.g. Gardner & Haddad 1953; Mountcastle 1957; Andersen et al. 1967; Clark et al. 1973). Direct evidence for the importance of joint receptors for position sense and kinaesthesia, as well as for the normal reflex coordination of muscle tone, has been demonstrated using selective removal of joint afferent inputs by local intra-articular injection of anaesthetics or by partial joint de-afferentation (Browne et al. 1954; Provis 1958; Freeman & Wyke 1966, 1967b; Ferrell et al. 1985, 1987; Gandevia et al. 1983; Clark et al. 1989). Thus, it seems reasonable to conclude that joint receptor afferents, in addition to provide the CNS with information about nociceptive/harmful stimuli, also contribute in more sophisticated tasks, such as in proprioception and muscle coordination.

In orthopaedic literature, many investigations have focused on the bony, ligamentous and vascular anatomy of different joints, while comparatively little attention has been devoted to the functional role of the articular neuronal structures (Kennedy et al. 1982). The latter might partly be due to early failures in attempts to elucidate the existence of ligamento-muscular protective reflexes (Andersson & Stener 1959; Stener 1959; Petersén & Stener 1959). The growing number of sports related joint injuries during the last decades, and the fact that the knowledge of optimal treatment programmes remains partly incomplete, have certainly played an important role in creating a somewhat renewed interest for the functional significance of joint receptor activity in the control of joint stability (cf. Kennedy et al. 1982; Clancy 1983; Noyes et al. 1984; Zimny 1988a).

Sensory endings in the knee joint

To a large extent our current knowledge of the morphology and function of articular sensory endings comes from studies performed on the cat and human knee joints. The first systematic investigations of the distribution and termination of nerves in the knee joint were performed in the 1940's by Gardner (1944, 1948). The histology of the knee joint, and the sensory endings within different structures (capsule, ligaments and menisci), have since then been the subjects of extensive investigations (for reviews, see Wyke 1981; Newton 1982; Zimny 1988a). Thus, morphological studies have revealed that the nerve endings may be classified into four categories: Ruffini endings, Pacinian corpuscles, Golgi tendon organ-like endings, and free nerve endings (according to Freeman & Wyke's (1967a) classification, type I, II, III and IV endings, respectively).

The Ruffini endings usually consist of a cluster of two to six thinly encapsulated globular corpuscles with a single myelinated parent axon. Several structural varieties of the Ruffini endings in different species and tissues have been reported (Halata 1977, 1988; Polácek 1966). Ruffini endings have been found in both the capsule and the medial meniscus of the knee joints of cat as well as man (Gardner 1944; Samuel 1952; Boyd 1954; Skoglund 1956; Polácek 1966; Freeman & Wyke 1967a; Halata 1977; O'Conover & McConnaughay 1978; Grigg & Hoffman 1982; Grigg et al. 1982; Halata et al. 1985; Zimny et al. 1988; Zimny 1988b). They have also been observed in the medial collateral ligament of the cat (Freeman & Wyke 1967a) and
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in the human anterior cruciate ligament (Zimny et al. 1986; Schutte et al. 1987). Recordings from Ruffini afferent fibres arising from the cat knee joint capsule have shown that these receptors are slowly adapting and have low mechanical thresholds (Boyd & Roberts 1953; Andrew & Dodt 1953; Boyd 1954; Skoglund 1956; Eklund & Skoglund 1960; Freeman & Wyke 1967a; Grigg & Hoffman 1982). According to their behavioural characteristics, these receptors may be categorized as static and dynamic mechanoreceptors, which (depending on the location of the receptor) are able to signal static joint position, intra-articular pressure and amplitude and velocity of movements (cf. Freeman & Wyke 1967a; Wood & Ferrell 1984; Ferrell 1987; Zimny 1988a). Furthermore, receptors tonically active at intermediate joint angles have been found both in the knee joint capsule and in the cruciate ligaments (Skoglund 1956; Ferrell 1980; see also Burgess & Clark 1969). It has been suggested that these 'mid-range' units may give information concerning joint angles and/or limb movements (see e.g. Ferrell 1980; Proske et al. 1988; Zimny 1988a), and it seems quite possible that an important part of the 'mid-range' afferents arise from Ruffini endings (cf. Skoglund 1956).

The Pacinian corpuscles are thickly encapsulated, conical corpuscles, which are rapidly adapting and have low thresholds to mechanical stress. They have been identified within the knee joint capsule and the medial meniscus of both humans and cats (Samuel 1952; Boyd 1954; Skoglund 1956; Hromada & Polácek 1958; Polácek 1966; Freeman & Wyke 1967a; Halata & Groth 1976; Halata 1977; O'Connor & McConnaughey 1978; Halata et al. 1985; Zimny et al. 1988; Zimny 1988b), and recently also in the human anterior cruciate ligament (Zimny et al. 1986; Schutte et al. 1987). These mechanoreceptors are inactive in the immobile joint and when the joint is rotated at constant speed, but become active at acceleration and deceleration (Freeman & Wyke 1967a; Zimny 1988a). Accordingly, Pacinian corpuscles are regarded solely as dynamic mechanoreceptors (Boyd 1954; Skoglund 1956; Freeman & Wyke 1967a; Zimny 1988a).

The largest of the articular mechanoreceptors are usually thinly encapsulated, fusiform corpuscles, that appear to be the articular homologue to the tendon organs of Golgi. In the knee joint the Golgi tendon organ-like endings were until recently believed to be confined to joint ligaments (i.e. the collateral ligaments (Andrew 1954; Skoglund 1956; Freeman & Wyke 1967a) and the cruciate ligaments (Boyd 1954; Skoglund 1956; Freeman & Wyke 1967a; Schultz et al. 1984; Zimny et al. 1986; Schutte et al. 1987)). However, in a recent investigation the medial meniscus of the human knee joint was also found to contain this type of mechanoreceptor (Zimny et al. 1988; Zimny 1988b). Electrophysiological data show that the Golgi tendon organ-like endings are slowly adapting, have high thresholds, and are completely inactive in immobile joints (Freeman & Wyke 1967a). It has been suggested that these receptors measure the tension of the ligaments (Andrew 1954; Skoglund 1956) most efficiently when the joint is at the extremes of the movement range, due to their high thresholds (cf. Freeman & Wyke 1967a; Wyke 1981; Zimny 1988a).

Free nerve endings are widely distributed throughout most of the articular tissues and constitute the articular nociceptive system (for reviews, see Wyke 1981; Proske et al. 1988; Zimny 1988a). These endings remain inactive during normal circumstances but become active when they are subjected to abnormal mechanical deformation or to chemical agents, for instance inflammatory mediators like histamine, bradykinin and
prostaglandin (Andrew & Dodt 1953; Freeman & Wyke 1967a; Burgess & Clark 1969; Coggeshall et al. 1983; Schaible & Schmidt 1983, 1985, 1988a,b; Grigg et al. 1986; Schaible et al. 1987a; Ferrell et al. 1988).

To summarize, it has been shown that the human as well as the cat knee joint capsules, ligaments and menisci possess sensory nerve endings which are morphologically different and have various response characteristics to mechanical stress. The sensory system within the articular tissues of the knee joint is therefore not only capable of detection of noxious stimuli, but also potentially able to provide the CNS with information about speed, acceleration, position and direction of movements.

Innervation of the knee joint

The knee joint of the cat is innervated by three nerves which contain afferent fibres primarily destined for articular tissues. These nerves are the posterior articular nerve (PAN), the medial articular nerve (MAN) and the lateral articular nerve (LAN). The PAN is the largest and most constant of the knee joint nerves. It arises from the posterior tibial nerve, usually just below the popliteal fossa, and innervates the posterior capsule, the posterior fat pads, the posterior oblique ligament, the medial and lateral collateral ligaments, the posterior cruciate ligament, and the posterior parts of the annular ligaments surrounding the lateral and medial menisci (Gardner 1944; Skoglund 1956; Freeman & Wyke 1967a). In some animals, afferent fibres from the popliteus muscle take their course in the PAN (McIntyre et al. 1978a). The MAN arises in the antero-medial region of the thigh as a branch of the saphenous and/or the obturator nerves. This articular nerve appears to be distributed to the medial and antero-medial aspect of the fibrous capsule, the medial collateral ligament, the meniscus, the ligamentum patellae, the infrapatellar fat pad, and the medial part of the patellar periostium (Gardner 1944; Skoglund 1956; Stener 1959; Freeman & Wyke 1967a). The most inconstant of the knee joint nerve is the LAN, which, when present, takes a recurrent course after branching from the common peroneal nerve and supplies the capsule of the superior tibio-fibular joint, the infero-lateral tissues of the knee joint, and the peroneal muscles (Gardner 1944; Skoglund 1956; Freeman & Wyke 1967a). In addition to the primary articular nerves (i.e. PAN, MAN and LAN), the knee joint is usually also innervated by a number of small intramuscular articular nerves, which arise within the substance of the muscles from branches of the main muscle nerves. Such accessory articular nerves have been found in the quadriceps, in the sartorius and in the gastrocnemius muscles (Freeman & Wyke 1967a). They may innervate the medial and posterior capsule, ligamentum patellae, the infrapatellar fat pad, and the upper end of the medial collateral ligament (Freeman & Wyke 1967a). Notably, while it is known that the capsule is innervated by several nerves and that the posterior cruciate ligament is innervated at least by the PAN, very little is known about the nerve supply of the anterior cruciate ligament.

Projections of knee joint afferent activity to supraspinal structures

Pathways from the knee joint afferents have an extensive divergence in the spinal cord (Craig et al. 1988). After entering the spinal cord, the sensory inflow from the knee joint is conveyed through different spinal and ascending pathways to motoneurones and supraspinal structures, including both the cortex and cerebellum. Information from knee joint afferents ascend in the dorsal columns and in the spinothalamic, spinoreticular, spinocervical and spinocerebellar pathways (Burgess & Clark 1969;
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Lindström & Takata 1972; Kuno et al. 1973; Fields et al. 1977; Maunz et al. 1978; Meyers & Snow 1982; Harrison & Jankowska 1984; Craig & Kniffki 1985). Experiments showing that knee joint receptor afferents may provide information that gain access to consciousness (Browne et al. 1954; Provins 1958; Gandevia et al. 1983; Clark et al. 1989), has bolstered the view that articular afferents provide the CNS with information relevant to movement and position sense (for reviews, see e.g. Goodwin 1976; Matthews 1977).

Reflex effects from knee joint afferents on skeletomotor neurones

Both electrical and physiological stimulation of joint receptor afferents have repeatedly been reported to evoke reflex effects on α-motoneurones. In early investigations, with graded electrical stimulation of knee joint afferents, it was found that α-motoneurones were influenced by activity in high threshold joint afferents, but not by activity in low threshold (below 2.0 times the nerve threshold) joint afferents (Cohen & Cohen 1956; Eccles & Lundberg 1959; Holmqvist 1961; Holmqvist & Lundberg 1961). These findings, together with the observation that most joint receptor afferents seem to be active only at or near the end of the joints' normal working ranges (Andrew & Dodt 1953; Skoglund 1956; Burgess & Clark 1969; McCall et al. 1974; Clark & Burgess 1975; Grigg 1975; Millar 1975; McIntyre et al. 1978a; Carli et al. 1979; Ferrell 1980; Grigg & Greenspan 1977; Rossi & Grigg 1982; Aloisi et al. 1988), have made it commonly believed that joint receptors are likely to act as "limit detectors", which regulate the excitability of the muscles and hence prevent hyperextension/hyperflexion of the joints (Lundberg et al. 1978; Baxendale & Ferrell 1981; Grigg & Hoffman 1982; Kennedy et al. 1982; Rossi & Grigg 1982; Schaible & Schmidt 1983; Zimny 1988a). Yet, the facts which constituted the basis for this simple view on the function of joint receptors has recently been somewhat modified by the observation that a considerable proportion of the knee joint afferents are 'mid-range' units (Ferrell 1980). Also, it has been shown that low threshold joint afferents may evoke reflex effects in α-motoneurones (cf. Lundberg et al. 1978).

In most studies with natural stimulation of joint receptor afferents, either mechanical stimulation of the capsule or rather nonspecific stimulation of different joint structures (mostly then also involving the capsule) has been employed. Many of these studies have been made with EMG used as the assessment technique, which is a relatively crude method. Yet, it has been observed for the knee joint as well as for the ankle joint, that activation of capsular receptors, by passive joint movements and by externally applied pressure, may induce reflex effects on muscles acting at these joints (Cohen & Cohen 1956; Andersson & Stener 1959; Ekholm et al. 1960; Grigg et al. 1978; Baxendale & Ferrell 1981; Baxendale et al. 1987, 1988; Freeman & Wyke 1967b). In investigations with direct recording from joint afferents, it has been shown that increased intra-articular pressure in the knee joint, which is believed to mainly stimulate receptors in the capsule, powerfully excites joint afferents (Andrew & Dodt 1953; Grigg et al. 1982; Wood & Ferrell 1984; Ferrell et al. 1986; Ferrell 1987). In yet other studies, with EMG or monosynaptic testing technique, it has been demonstrated, that increased intra-articular pressure in the knee joint may elicit inhibitory reflex effects on α-motoneurones projecting to muscles around the joint (Ekholm et al. 1960; deAndrade et al. 1965; Spencer et al. 1984; Iles et al. 1984; Fahrer et al. 1988; Wood et al. 1988). It has also recently been reported that receptor activation due to acute knee joint inflammation (experimentally induced by intra-articular
injection of carrageenan and kaolin) can alter the magnitude of the flexion withdrawal reflex of cat knee joint flexors (Ferrell et al. 1988; see also He et al. 1988).

The role of ligamentous sensory endings in the reflex regulation of the muscle activity seems to be of particular clinical relevance in the light of the importance of the neuromuscular system for maintaining functional joint stability (Goldfuss et al. 1973; Markolf et al. 1976; 1981; Noyes et al. 1984; Baratta et al. 1988). Unfortunately, compared to the information available on effects elicited from capsular receptors, the knowledge about reflex effects evoked by natural stimulation of sensory endings in ligaments is extremely sparse. In a few studies with EMG or monosynaptic testing technique, it has been reported that the activity in the hamstrings and the quadriceps muscles might be altered by increased tension in the medial collateral ligament (Palmer 1958; Ekholm et al. 1960) or in the anterior cruciate ligament (Solomonow et al. 1987). However, some of these authors (Palmer 1958; Ekholm et al. 1960) do not explicitly account for the loads used, while others (Solomonow et al. 1987) used quite forceful stretches of the ligaments. There are also conflicting studies (Andersson & Stener 1959; Stener 1959; Petersén & Stener 1959; Pope et al. 1989; Grabiner et al. 1989) demonstrating that ligament stretches do not exert much influence directly on the skeletomotor system, at least at moderate loads. Thus, although some information is available, further studies have to be performed in order to clarify the function of the ligamentous sensory systems in the reflex regulation of the muscle activity.

Reflex effects from knee joint afferents on fusimotor neurones

During the 1960's, important contributions to our knowledge of the morphology and physiology of articular sensory systems were made by Freeman and Wyke. In addition to their results on the behavioural characteristics of joint receptors (Freeman & Wyke 1967a), these authors found, that mechanical stimulation of articular structures caused 'reflex changes in motor unit activity in the leg muscles' (Freeman & Wyke 1967b), and further, that partial denervation of the cat knee joint produced specific and persistent changes in posture and gait (Freeman & Wyke 1966). Based on these findings they concluded that reflexes from articular mechanoreceptors are 'significantly involved in the normal reflex coordination of the muscle tone in posture and movement', and proposed, on indirect evidence, that these receptors 'operate polysynaptically via the gamma motoneurone loop' (Freeman & Wyke 1967b; Wyke 1981; cf. also Ekholm et al. 1960).

The first observation that joint receptor afferents in fact can affect the activity of γ-fibres was made by Voorhoeve & van Kanten in 1962. Some years later, with micro-electrode recordings from unclassified γ-motoneurones, Grillner et al. (1969) found that these cells could be excited as well as inhibited during stimulation of high threshold PAN afferent fibres. Moreover, with indirect evaluation of fusimotor reflex effects by recording from primary muscle spindle afferents, McIntyre et al. (1978b) have showed that electrical stimulation of the PAN might activate static fusimotor neurones. Recently, it was also shown that nonspecific natural activation of knee joint afferents from the ipsilateral (He et al. 1988) as well as from the contralateral hind limb (Appelberg et al. 1979, 1981) can change the activity in fusimotor neurones.

Thus, it is known that activation of joint afferents may induce reflex effects on the γ-muscle-spindle system. However, in order to elucidate the functional role of joint reflexes on the γ-muscle-spindle system, and thereby also the validity of Freeman and
Wyke's hypothesis, a number of questions remains to be answered. For instance, how common and potent are the fusimotor reflex effects evoked by joint afferents? Are static and dynamic $\gamma$-motoneurones influenced similarly or differently during activation of joint afferents? How many synapses are there in the different reflex pathways from the joint nerves to the $\gamma$-motoneurones? How do low and high threshold joint afferents affect the $\gamma$-motoneurones? May natural activation of receptor afferents from different specific joint structures (e.g. capsule and ligaments) influence the $\gamma$-motoneurones potently enough to significantly alter the activity in the muscle spindle afferents? If the spindle afferents are influenced, are then both the dynamic and static sensitivity affected. Are there any differences as to how spindle afferents from different muscles are influenced?

The aims of the present investigation

The general object of this series of investigations was to study reflex effects on the $\gamma$-muscle-spindle system evoked by natural and electrical stimulation of articular receptor afferents, and thereby investigate hypotheses that joint receptor afferents contribute to the normal regulation of muscle tone through reflexes on the $\gamma$-muscle-spindle system (Freeman & Wyke 1967b) and that this mechanism may serve to regulate joint stiffness and joint stability (Paper I). The specific aims can be summarized in the following points:

- With micro-electrode recordings from a large sample of $\gamma$-motoneurones (classified as static or dynamic) and with graded electrical stimulation of the PAN (Paper I), examine (i) the responsiveness (i.e. number of cells with effect/number of cells tested), (ii) the occurrence of excitatory and inhibitory reflex effects, and (iii) the thresholds and (iv) segmental latencies for the effects evoked.

- With recordings from single GS primary muscle spindle afferents (Paper II), (i) investigate whether natural (pressure on capsules and joint movements) and electrical stimulation of hind limb joint receptor afferents can induce significant changes in muscle spindle afferent responses, via reflex actions on fusimotor neurones, and (ii) clarify and compare the types and sizes of the fusimotor reflex effects evoked by stimulation of ipsi- and contralateral joint receptor afferents.

- Develop a new technique which permits assessment of simultaneously evoked fusimotor reflexes on several single muscle spindle afferents from the same or different muscles (Paper III). This was deemed important in order to examine and compare the fusimotor reflex effects evoked on different spindle afferents in absence of the biasing action caused by spontaneous variations in the descending fusimotor drive and in the setting of the spinal interneuronal network.

- With histological technique investigate the cat cruciate ligaments with regard to the occurrence of different types of sensory endings (Paper V), and thereby disclose the types of receptors that might be activated by increased tension in these ligaments.

- With the method described in Paper III, (i) investigate whether natural stimulation (i.e. increased tension) of the PCL (Papers IV and VI) and the ACL (Paper VII) may evoke fusimotor reflex effects, which are potent enough to cause changes in the activity of muscle spindle afferents from a flexor (PBSt) and an extensor (GS) muscle acting at the knee joint, and (ii) reveal and compare the types and sizes of the fusimotor reflex effects evoked by stimulation of the PCL and the ACL on GS and PBSt muscle spindle afferents.
METHODS

Paper I

This report is concerned with reflex effects on static and dynamic hind limb \(\gamma\)-motoneurones evoked by graded electrical stimulation of knee joint afferents. A full account of the general methods employed has been given in previous papers (Johansson 1981; Appelberg et al. 1983a). This section is therefore mainly restricted to methodological aspects related to the identification and classification of \(\gamma\)-motoneurones, to the preparation and stimulation of the PAN, and to the estimation of the latencies and the thresholds of the evoked reflex effects.

Preparation

The experiments were performed on \(\alpha\)-chloralose anaesthetized cats. The experimental setup is shown in Fig. 1. Two laminectomies were always made. One of them exposed the lower thoracic cord (Th\(_{12}\) - Th\(_{13}\)) where the dorsal column and the entire right half of the spinal cord were removed over a distance of about 10 mm at the Th\(_{13}\) level (i.e. semi-spinalization). The other laminectomy was performed to expose the spinal cord at the L\(_{4}\) - S\(_{1}\) level. In addition to these laminectomies, two craniotomies were made, one over the right mesencephalon (for electrical stimulation of the rubral region), and the other over the posterior part of the cerebellum (for surface recording from the left paramedian lobule). A number of muscle nerves in the left hind limb were dissected and prepared for electrical stimulation.

Identification and classification of the \(\gamma\)-cells

Extra-, juxta- and intracellular micro-electrode recordings were made from single ventral horn cells. A cell was identified as a \(\gamma\)-motoneurone when an antidromic all-or-nothing potential, with fixed latency, could be evoked by stimulation of one particular muscle nerve, and when the conduction velocity of the axon was below 55 m/s (cf. Leksell 1945; Kuffler et al. 1951; Eccles et al. 1960). Most of the \(\gamma\)-motoneurones were classified as static or dynamic using the indirect method of mesencephalic stimulation (electrical stimulation of the rubrobulbospinal path in the MesADC is known to exert selective influence on dynamic \(\gamma\)-motoneurones, for details of this classification method, see Jeneskog & Johansson 1977; Appelberg 1981; Johansson 1981; Appelberg et al. 1982a). Stimulation of the mesencephalon was performed via two separate sets of stereotaxically guided glass-insulated platinum-iridium electrodes. A selective activation of the rubrospinal tract (NR) was obtained by stimulation of the interposito-rubral fibres (Baldissera et al. 1972). Stimulation of presynaptic interpositorubral fibres, rather than of rubral cells, was indicated by a prolongation of the latency of the rubrospinal volley recorded on the dorsolateral aspect of the thoracic spinal cord (DLF). The rubrobulbospinal path was stimulated in the MesADC-region, which includes the dorsocaudal part of the red nucleus and the region caudal and just dorsal to that structure (Jeneskog & Johansson 1977; Appelberg 1981). The position of the MesADC electrodes was judged as satisfactory when, with a short-train stimulation, a typical ‘staircase’ shaped volley could be recorded from the DLF in absence of a rubrospinal volley, and when, at the same time, a short latency (below 8.5 ms) D\(_{1}\) -
Fig. 1. Schematic picture showing the different stimulation and recording sites (Paper I). Reflex effects evoked by single-shock stimulation of the PAN (Stim. PAN) were assessed with micro-electrode recordings from single ventral horn cells (Rec. \( \gamma \)-cells). Each cell was identified as a \( \gamma \)-motoneurone according to the conduction velocity of its axon calculated from the latency of the antidromic response induced by electrical muscle nerve stimulation (Stim. muscle nerves). Classification of the \( \gamma \)-cells as static and dynamic was obtained using the indirect method of mesencephalic stimulation (Stim. MesADC). The correct placement of the MesADC electrodes was achieved by checking that stimulation in the MesADC evoked a rubrobulbospinal volley, recorded on the dorsolateral aspect of the thoracic spinal cord (Rec. DLF), but not a rubrospinal volley, while stimulation of the rubrospinal tract (Stim. NR) gave a rubrospinal but no rubrobulbospinal volley, and by recording the latency for the appearance of the climbing fibre response on the paramedian lobule of the posterior lobe of cerebellum (Rec. PM). The incoming PAN volley to the dorsal root entry zone was recorded (Rec. DR) in order to determine the thresholds for the evoked reflex effects and for calculation of the segmental latencies. The threshold for the most excitable fibres in the PAN was assessed by recording from the sciatic nerve (Rec. sciatic nerve). For further details, see text. (Modified from Johansson 1988)
zone climbing fibre response appeared at the surface of the left paramedian lobule (PM) of the posterior lobe of the cerebellum. Special care was always taken to confirm that, while a rubrospinal volley was elicited by rubral stimulation, no such volley was evoked by the rubrobulbospinal stimulation.

**Stimulation of the PAN**

As a standard routine, the dissected and cut central end of the PAN was subjected to single-shock stimulation at 1-2/s. The intensities of the stimulations were always graded in multiples of the stimulation strength (1.0 T) at which an early negative cord dorsum potential appeared (cf. Holmqvist 1961; Carpenter et al. 1963; Lundberg et al. 1978), recorded by a monopolar silver ball electrode on the dorsal root entry zone (DR). The early negative cord dorsum potential was found to correspond to 1.2-1.3 times the threshold for the most excitable fibres in the PAN, determined by recording from the sciatic nerve. Assessment of the thresholds for the responses was performed by changing the intensities by steps of 0.05-0.10 T.

**Measurements of response latencies**

The latencies were assessed from a series of responses at each stimulation intensity. First the latency from the stimulus artifact to the appearance of the cell response was measured. From this value the peripheral conduction time (i.e. from the stimulation electrode to the recording electrode at the dorsal entry zone), for the fastest conducting joint nerve afferents, was subtracted (assumed conduction velocity 90 m/s, cf. Boyd & Davey 1968; Burgess & Clark 1969; Lundberg et al. 1978; Ferrell 1980). Thus, each latency value represents the time from the presumed arrival of the activity in the fastest joint afferents at the site of the dorsal root entry zone electrode to the onset of the cell response.

**Data processing and analysis**

The cell responses and cord dorsum potentials were stored on magnetic tape, and all analysis was performed off-line. These data were then superimposed on an oscilloscope display, and the averaged responses and potentials were calculated by aid of an averaging computer and a desktop calculator, and plotted on a X-Y plotter (for details, see Appelberg et al. 1983a). Thus, all measurements were made on the averaged records. The occurrence of excitatory and inhibitory reflex effects was evaluated on the basis of conventional criteria.

**Papers II, III, IV, VI and VII**

In these papers the same general method for the recording of reflex induced alterations in the sinusoidal response of muscle spindle afferents was used. A brief summary of the general method and specific methodological aspects related to the separate investigations are given in this section. For a detailed account of the anaesthesia, preoperative care, setup and preparation, see Appelberg et al. (1981, 1982b, 1984, 1986).

**Animals and preparation**

The experiments were performed on cats anaesthetized with α-chloralose. Arterial blood pressure, end-expiratory CO\(_2\) and temperature were continuously monitored,
and kept within the physiological limits throughout the experiments. Arterial blood samples were taken regularly for analysis of the acid base status on a blood gas analyzer. The arterial pH was kept above 7.39, if necessary by infusion of sodium chloride acetate or of sodium bicarbonate solutions.

The ipsilateral hind limb. In the ipsilateral hind limb conventional nerve-muscle preparations were made for lateral gastrocnemius, plantaris and soleus muscles (GS), and for posterior biceps and semitendinosus muscles (PBSt). The medial gastrocnemius was routinely removed. For the muscles prepared, resting lengths and maximum physiological lengths were determined in situ and marked by appropriate reference labels in the surrounding tissues. The tendons were then disconnected from their points of insertion and tied to two separate stretching devices. Apart from the GS, the PBSt and the knee joint nerves, the ipsilateral hind limb was extensively denervated. However, the tibial nerve was left intact, in order to permit natural stimulation of sensory endings in the capsule and in the cruciate ligaments. The femoral nerve was left intact in the experiments in which reflex effects evoked from receptors in the ACL were studied, and in one of the experiments in which the PCL was stimulated. In some of the experiments (Paper II), the PAN and/or the sural nerve were dissected free and cut distally, so that their proximal ends were accessible for electrical stimulation (cf. Skoglund 1956).

To get access to the cruciate ligaments, an anterior midline incision was made just below the patella. The intra-articular fat pad was removed. When the PCL was prepared it was necessary to cut the ACL. A metal wire or a braided thread was passed around the PCL or the ACL (cf. Stener 1959), so that a traction force could be applied later on during the experiment (see below: Reflex stimulations).

The contralateral hind limb. This limb was either allowed to remain intact throughout the experiment, operated on during the course of the experiment, or operated on at the beginning of the experiment. In the latter experiments, the GS and the PBSt muscles were prepared with intact nerve supply and, with exceptions for these nerves and the femoral nerve, the contralateral limb was denervated to the same extent as the ipsilateral one. In three of these experiments, the PAN was dissected, cut and prepared for electrical stimulation (cf. Skoglund 1956). In some of the animals, in which the contralateral limb was left intact initially, attempts were made to investigate the specific contribution from contralateral joint afferents to fusimotor reflexes evoked by manipulation of the intact limb (Paper II). This was performed by interfering with different categories of receptor afferents (i.e. muscle, cutaneous and joint afferents), either surgically by cutting nerves or tendons or by removal of the skin, or functionally by intra-articular injections of local anaesthetics (see below: Reflex stimulations).

Fixation of the limbs. The ipsilateral hind limb was always carefully immobilized with pairs of horizontal pins inserted into the femur and the proximal and distal parts of the tibia. In the experiments in which the contralateral limb was prepared, this limb was fixed in the same way as the ipsilateral one. When the contralateral limb was left intact, this limb was pinned to the metal frame only at the pelvis, so that the hip, knee and ankle joint could be easily manipulated. The fixations were made in order to prevent any mechanical interactions between the reflex stimulations and the ipsilateral GS or PBSt muscles.
The spinal cord. A laminectomy exposing the spinal cord from Th12 to S1 was regularly performed, and some of the animals were spinalized at Th12, L3 or L4, either during the initial operation or later on during the course of the experiment.

Reflex stimulations

Paper II. In this investigation both natural and electrical stimulation of ipsi- as well as contralateral joint receptor afferents were made. The natural stimulations used included: (i) tonic pressure applied to the latero-frontal aspects of the ipsi- and contralateral knee joint capsules (cf. Skoglund 1956; Ekholm et al. 1960; Schaible et al. 1986; Baxendale et al. 1987), (ii) tonic pressure applied to the lateral aspect of the contralateral ankle joint, and (iii) manually performed extension or flexion (up to maximum joint angles) of the contralateral hip, knee and ankle joints together, or separately (cf. Skoglund 1956; Burgess & Clark 1969; Grigg 1975; Rossi & Grigg 1982; He et al. 1988). In the experiments in which significant reflex effects were elicited by natural stimulation, attempts were made to assess the actual contribution from joint receptor afferents. This was achieved by selectively abolishing the stimulation induced activation of joint receptors with intra-articular injections of 0.2-0.4 ml lidocaine hydrochloride (Xylocard: 200 mg/ml, Hässle; Xylocain: 40 mg/ml, Astra) (cf. Clark et al. 1979; Ferrell 1980; Baxendale & Ferrell 1985).

The electrical stimulation of the ipsi- and contralateral PAN was performed with bipolar silver hook electrodes. Repetitive stimulation at 40 and 100 Hz was used and the intensities of the stimuli were graded in multiples of the stimulation threshold (T) for the most excitable fibres running in the PAN (1T), determined by recording from the sciatic nerve.

Paper III. The reflex stimulations in this study consisted of (i) tonic stretch of the ipsilateral GS and PBSt muscles, (ii) repetitive electrical (40 Hz) stimulation of the ipsilateral sural nerve with different stimulation intensities, (iii) extension of the contralateral hip, knee and ankle joints together, (iv) flexion of the contralateral knee and ankle joint together, (v) flexion and extension of the contralateral ankle joint, and (vi) tonic pressure on the frontal aspect of the contralateral knee joint applied distally to the patella.

Papers IV and VI. Receptor afferents in the ipsilateral PCL were stimulated by pulling the metal wire which was tied around the ligament. The standard traction forces applied were 5-40 N (measured with a dynamometer). On occasions, gradually increased loads ranging from 5 to 40 N were used in order to assess the dependence between the size of the fusimotor effect evoked and the size of the load applied.

Paper VII. The stimulation of the ACL was performed in an almost identical way to that of the PCL. The only differences were that a braided thread was used instead of a metal wire and that traction forces up to 70 N sometimes were used.

All different types of natural stimulations were performed as standardized and reproducible sequences, always applied 10-15 s before and maintained during the data sampling.
Stimulation of the receptor bearing muscles

The ipsilateral GS and PBSt muscles, which were connected to electromagnetic pullers (in Paper II, only the GS muscle), were continuously stretched and released (between 10 and 2 mm below the maximum physiological length) using ramp-and-hold command signals (velocity of stretch = 10 mm/s; plateau duration = 15 s). Repetitive sinusoidal stretches at 1 Hz for the GS and 0.9 Hz for the PBSt muscles were superimposed on the plateaus of the ramp-and-hold stretches (cf. Fig. 2A).

Recording and analysis

Recording. Functionally single muscle spindle afferents were dissected from cut dorsal root filaments and placed on silver-wire recording electrodes. To discriminate between spindle afferents and Golgi-tendon organs, twitch-tests (Matthews 1933; Hunt & Kuffler 1951; Matthews 1972) were performed using maximal contractions. The afferents were classified as primary or secondary spindle afferents on the basis of their conduction velocity (division line at 72 m/s, cf. e.g. Boyd & Davey 1968; Hulliger 1984).

Any change of fusimotor activity was assessed indirectly, by monitoring the responses of functionally single spindle afferents to sinusoidal stretching of the receptor bearing muscles. In the study accounted for in Paper II only one GS muscle spindle afferent was recorded from on every occasion. This recording technique was then further developed (Paper III), so that recordings could be made simultaneously from 2, 3 or 4 single muscle spindle afferents from a flexor (PBSt) and/or an extensor (GS) muscle (Papers III, IV, VI and VII). Recordings of afferent responses to sinusoidal stretches were only made during the plateau phase (at 2 mm below the maximum physiological muscle length). The methods used for recording, processing and analysis of primary spindle afferent responses are shown in Fig. 2.

Processing of afferent responses. Changes in fusimotor effects were assessed by comparing control and test responses. The control responses were recorded in absence of any intentional reflex stimulation, whereas the test responses were obtained during ongoing stimulation of joint receptor afferents (see above, Reflex stimulations). The instantaneous firing frequency was calculated from the afferent responses and displayed on the oscilloscope (Fig. 2B). The afferent responses were averaged for 10 successive sinusoidal stretching cycles (Fig. 2B), and the averaged responses were displayed as cycle histograms, which show the probability density of firing throughout the sinusoidal stretch cycle (Fig. 2B). To the histograms simple sinusoids were fitted by the use of a least square algorithm method which ignored periods of afferent silence (Hulliger et al. 1977a). Both the averaging and the fitting of the sinusoids were performed on-line with a NORD computer.

Analysis. The following parameters of the fitted sines were taken as quantitative estimates of the afferent responses (Fig. 2C); (i) the mean rate of discharge ("fitted mean"), (ii) the depth of modulation, and (iii) the phase. These response parameters for a number of control-test pairs were sampled in order to assess the stability and degree of consistency of the evoked changes in fusimotor activity. Thus, for each unit and for every control-test pair, the numerical values of the fitted mean, the depth of modulation and the phase, were stored on files for off-line statistical analysis and construction of scatter diagrams (Fig. 2C).
CONTROL MEASUREMENT  TEST MEASUREMENT

A RECORDINGS

Afferent discharge (1 mV)

Muscle length (mm)

Force (2 N)

Time (s)

B DIGITAL PROCESSING OF AFFERENT RESPONSES

Instantaneous firing rate computation

Averaging of ten cycles and fitting of simple sinusoids

C ANALYSIS

Assessment of fusimotor effect

Change in:
- fitted mean
- depth of modulation
- phase

Change in fitted mean (imp/s)

Change in modulation (imp/s)

Change in phase (deg)
Assessment of fusimotor effects. The criteria used in this study for classification of fusimotor reflex activation are based on earlier investigations with controlled electrical stimulation of functionally single γ-efferents (Crowe & Matthews 1964a,b; Appelberg et al. 1966; Matthews 1972; Hulliger et al. 1977a,b; Emonet-Dénand et al. 1977; Hulliger 1979; Appelberg et al. 1982b). The occurrence of predominantly dynamic fusimotor reflex activation was inferred when there was (1) a clear increase in depth of modulation of the fitted sine of the test responses compared to the control responses, combined with (2) a moderate increase in fitted mean, and (3) occurrence of afferent silence during the release phase of the sinusoidal stretching (cf. Fig. 2C).

An increase in predominantly static fusimotor activity was inferred when there was (1) an appreciable decrease in depth of modulation of the fitted sine of the test response, combined with (2) a sizable increase in fitted mean, and (3) an absence of afferent silence during the release phase of the sinusoidal stretching.

An increase in mixed (both static and dynamic) fusimotor reflex activation was inferred when there was (1) a relatively small change in depth of modulation, combined with (2) a large increase in fitted mean, and (3) absence of afferent silence during the release of sinusoidal stretching.

Moreover, the type and size of the evoked fusimotor reflex effects were also estimated quantitatively by comparing the reflexly induced changes in the fitted sine characteristics (test values minus control values for fitted mean and depth of modulation) to the changes known to be evoked in responses from GS primary spindle afferents by selective electrical stimulation of single static, of single dynamic and of both static and dynamic fusimotor fibres. Based on the data from Hulliger et al. (1977b) calculations were made (Appelberg et al. 1982b) of the slopes of the reference lines used for these comparisons (labeled $\gamma_S$, $\gamma_S + \gamma_D$ and $\gamma_D$, see the scatter diagram in Fig. 2C).

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Fig. 2. Schematic diagram illustrating the methods used for recording, processing and analysing the sinusoidal responses of single muscle spindle afferents (Paper II, III, IV, VI and VII). A: simultaneous recordings of the muscle spindle afferent discharges (exemplified by a recording from a primary spindle afferent from the GS muscle), the muscle force, and muscle length (in relation to the maximum physiological length, which represents 0 mm) were performed. B: the instantaneous firing frequency was calculated on-line. For both control and test conditions, responses to 10 successive sinusoidal stretching cycles were averaged. The averaged control (left-hand histogram) and test (right-hand histogram: increased tension of the ACL was used as test) responses were plotted against the phase of the stretch cycle, and simple sinusoids were fitted to each histogram. C: the mean rate of discharge (fitted mean), the amplitude (depth of modulation), and phase of the fitted sines were taken as quantitative estimates of the responses. The numerical values of these parameters, for each pair of control and test responses, were stored for off-line statistical analysis and construction of scatter diagrams. The assessment of the type of induced fusimotor reflex effect (i.e. static, dynamic or mixed static and dynamic) was deduced on the basis of earlier investigations with controlled electrical stimulation of static and dynamic γ-fibres. In the scatter diagram, the slope of the line labeled $\gamma_D$ shows the ratio (1.07) of the changes in modulation/changes in fitted mean found with selective electrical stimulation of dynamic fusimotor fibres projecting to the GS muscle. The line labeled $\gamma_S$ (ratio -0.47) shows the corresponding changes obtained with selective electrical stimulation of static GS fusimotor fibres and the line labeled $\gamma_S + \gamma_D$ (ratio 0.24) the changes evoked by combined stimulation of static and dynamic fusimotor fibres (see Appelberg et al. 1982b, where data from Hulliger et al. 1977b were recalculated and replotted). For further details, see text.
Skeletomotor activity. In most of the experiments (Papers III, IV, VI and VII), the occurrence of any skeletomotor activity was detected by observing the display of the muscle force signals (recorded via the electromagnetic pullers: lowermost detection limit = 0.01 N) on the oscilloscope (Fig. 24). In some experiments the EMG activity from the GS and PBSt muscles was recorded, using needle or surface electrodes (Papers II and III). With the latter type of electrodes, large surface areas were recorded from, while needle electrodes were used to gain access to muscle fibres lying in the deeper portions of the muscle.

Paper V

This histological investigation was undertaken in order to determine which types of sensory endings the cat cruciate ligaments contains.

Preparation

Seven cruciate ligaments (4 PCL and 3 ACL) from the knee joint of adult pentobarbitone sacrificed cats were used. The cruciate ligaments were carefully dissected immediately after sacrificing the cats. In order to permit histological investigation also of the cartilage and bone attachments of the ligaments, these were prepared together with the tibial and the femoral condyles. Usually the fresh ligament-bone specimens were frozen in normal saline solution for storage and further processing. Before staining the frozen tissue samples were thawed and decalcified in 40% formic acid in vacuum.

Staining

The ligament-bone specimens were stained en bloc using the modification (Zimny et al. 1985) of the gold chloride method described by Gairns (1930). This staining technique was originally applied for demonstrations of nerve endings in skeletal muscle (Gairns 1930; Carey 1942; Cole 1946; Boyd 1962), but has recently, in the modified form, been used with success in studies made on the human cruciate ligaments, menisci and temporomandibular articular disks (Zimny et al. 1986; Schutte et al. 1987; Zimny 1988b; Zimny et al. 1988). The modifications introduced by Zimny et al. (1985) consist of differences in staining time for the formic acid and the gold chloride, and the use of frozen sections instead of teased tissue. After staining, the bone-ligament preparations were frozen and serially sectioned longitudinally at 10-20 μm on a freeze-microtome. Each section was mounted on a slide, dehydrated, covered with alcoholic gelatin, and then overlaid with a cover-slip.

Classification of the sensory endings

The serially sectioned specimens were investigated with a light microscope and photographed. The nerve endings were classified according to the receptors previously demonstrated in the capsule, collateral ligaments and menisci of the cat (Gardner 1944; Boyd 1954; Skoglund 1956; Poláček 1966; Freeman & Wyke 1967a; O'Connor & McConnaughey 1978; Grigg & Hoffman 1982). Despite the fact that the structure of the endorgans varied to some degree, it was usually possible to categorize them by carefully investigating the serial sections.
RESULTS

Paper I

Reflex effects evoked on 71 \( \gamma \)-motoneurones in 21 animals to electrical stimulation of the PAN are described in this paper. Thirty-one of the \( \gamma \)-cells were recorded from intracellularly, 18 juxtacellularly and 22 extracellularly. The majority of the \( \gamma \)-cells (67 out of 71) were classified as either dynamic or static using electrical stimulation of the MesADC (Appelberg 1981; Johansson 1981; Appelberg et al. 1982a). The \( \gamma \)-motoneurones projected to the following muscles; 24 to the PBSt (13 dynamic (D), 10 static (S) and one non-classified (N)), 33 to the GS (20 D, 12 S and 1 N), 8 to the ABSm (1 D, 6 S and 1 N), 4 to the DP (2 D, 1 S and 1 N), and 2 to Tib (1 D and 1 S).

Responsiveness. A high general responsiveness (i.e. number of cells with effect/number of cells tested) was observed for the whole sample of \( \gamma \)-cells. Thus, the overall responsiveness figures to stimulation intensities up to 107, were 91.9% for the dynamic and 93.3% for the static cells.

Thresholds for the effects. For all subpopulations of \( \gamma \)-cells (i.e. dynamic and static, flexor and extensor cells) excitatory as well as inhibitory reflex effects were found at both low and high stimulation intensities. Low threshold effects (i.e. effects evoked at intensities below 1.67) were observed in 70% of a total sample of 40 cells, for which the thresholds of the effects were investigated. The mean stimulation intensity (threshold) needed to provoke excitatory effects was very similar to that needed to evoke inhibitory effects, i.e. 1.41 \( T \pm 0.31 \) (S.D.) \((n = 29)\) and 1.43 \( \pm 0.61 \) \((n = 11)\), respectively.

Frequency of excitatory and inhibitory effects. The responsiveness and the frequency of excitatory and inhibitory reflex effects in 23 PBSt and 32 GS \( \gamma \)-motoneurones are shown in Fig. 3. Among the PBSt cells excitation was predominant, irrespective of whether they were dynamic or static, while for the GS cells excitatory and inhibitory effects occurred with an approximately equal frequency. For four of the GS \( \gamma \)-cells mixed effects (i.e. mixture of excitation and inhibition) were encountered.

Latencies and synaptic coupling. The segmental delays for 32 excitatory and 13 inhibitory effects were determined (see METHODS for details about the calculation of the latencies). Comparison between static and dynamic \( \gamma \)-cells, as well as between flexor and extensor cells, revealed no significant differences as regards the latencies for the evoked effects. The shortest latencies (2.8 ms) for excitatory effects were clearly compatible with a di- or trisynaptic pathway, while the fastest route for inhibitory effects seemed to be one synapse longer (shortest latency = 3.5 ms).

Variability in reflex effects evoked on the individual \( \gamma \)-cells. The individual \( \gamma \)-motoneurones of the present study showed considerable differences in the reflex effects evoked from the PAN. Thus, for any given cell, the type (excitation and/or inhibition), the threshold or the latency of the effects evoked, could not be predicted either from the allocation of the cell to a certain population or from the character of the ef-
Paper II

The main purpose of this study was to investigate whether natural and electrical activation of ipsi- and contralateral hind limb joint afferents evoke reflex effects on fusimotor neurones which are potent enough to significantly change the discharge of primary muscle spindle afferents. Responses of single primary muscle spindle afferents from the GS muscles of 28 cats were investigated during sinusoidal stretching of the receptor bearing muscle (GS). By comparing control (in absence of any joint stimulation) and test (during ongoing joint stimulation) responses it was possible to determine the occurrence, type and size of the induced fusimotor reflex effects.

Reflex effects evoked by electrical stimulation of the PAN. Electrical stimulation of the PAN was used in this investigation in order to compare the fusimotor reflex effects elicited on primary spindle afferents with those evoked on $\gamma$-motoneurones (Paper I). One of the 2 primary spindle afferents, which were studied with electrical stimulation of the ipsilateral PAN, showed reflex effects compatible with predominantly dynamic...
fusimotor activation, while the changes in the responses for the other afferent indicated activation of predominantly static fusimotor neurones. For both these afferents the effects appeared at stimulation strengths of 2.0T. For 7 primary spindle afferents the effects evoked by electrical stimulation of the contralateral PAN were investigated. Only one of these afferents was significantly influenced. This afferent exhibited excitatory dynamic fusimotor effects with a stimulation intensity as low as 1.5T.

Reflex effects evoked by natural stimulation of joint afferents. A total number of 45 primary spindle afferents were investigated with pressure applied to the ipsilateral knee joint capsule. Five of these were reflexly influenced. Of these 5 afferents, 3 exhibited inhibition of both static and dynamic fusimotor drive, one showed inhibition of predominantly dynamic fusimotor drive, and one was activated via reflex effects on dynamic fusimotor neurones.

Altogether, 22 primary afferents were investigated with contralateral knee joint pressure used as the reflex stimulus. In this sample, 5 afferents showed excitatory dynamic fusimotor reflex effects, one afferent showed mixed static and dynamic effects, while 16 afferents were without significant reflex effects to this type of stimulation. Two of the afferents, which exhibited reflex effects to stimulation of the contralateral knee joint, were found to be similarly influenced by pressure applied to the contralateral ankle joint.

For 9 primary afferents, which showed clear-cut fusimotor effects to flexion and/or extension of the intact contralateral hind limb, evidence was obtained for the contribution of joint receptor afferents to the evoked fusimotor reflexes. This was achieved by interfering, either surgically or with injections of local anaesthetics (see METHODS), with different afferent inputs (i.e. from muscle, cutaneous or joint receptor afferents) while extension or flexion of contralateral joints were used as reflex stimulation. Out of these 9 afferents, 7 showed predominantly dynamic fusimotor effects, while 2 exhibited mixed static and dynamic fusimotor effects.

The reflex nature of the effects. There are four lines of evidence which suggest that the changes in the sensitivity of muscle spindle afferents observed in this investigation were due to true fusimotor reflexes rather than to some accidental mechanical interactions between the stimuli and the receptor bearing muscle. First, the ipsilateral hind limb was always firmly immobilized, by pins inserted into the bones of the pelvis, femur and tibia (cf. METHODS). Secondly, most units investigated showed a considerable response variability in spite of identical mechanical stimulation. Thirdly, deepening of the general anaesthesia, or injecting a local anaesthetic into the synovial cavity of the joint, either abolished or greatly reduced the effects. Fourthly, fusimotor effects observed during mechanical stimulation of a particular joint disappeared when the joint nerves were cut. Furthermore, in 14 of 16 investigated animals, the fusimotor effects elicited by activation of joint afferents were not accompanied by EMG activity in the receptor bearing muscle, which indicates that the reflexes were mediated via γ-motoneurones rather than by β-motoneurones.

Paper III

A prominent finding in the investigations performed at our laboratory on the segmental reflex control of lumbar fusimotor neurones has been the great amount of
variability in the reflex effects evoked by different reflex inputs on the individual γ-cells and spindle afferents (Papers I and II; Appelberg et al. 1982b, 1983a,b,c,d, 1984, 1986; Johansson 1981, 1985, 1988; Johansson & Sojka 1985; Sojka 1985; Johansson et al. 1989). These observations indicate that fusimotor neurones have wide and individualized receptive profiles. However, since the studies referred to above were made on single γ-cells or on single spindle afferents, recorded from one at a time, the variability in fusimotor reflex effects obtained might partly have been due to spontaneous fluctuations of descending fusimotor drive and/or to alterations in the setting of the spinal interneuronal network. In order to clarify this issue, and to further elucidate the complex organization of the interneuronal network projecting to the fusimotor neurones, a new technique was developed. This method, which is introduced in this paper, permits simultaneous assessment of changes in the responses of several single muscle spindle afferents, and thereby investigation of different spindle afferents during identical descending and segmental conditions.

In this study, sets of single spindle afferents from the GS and/or the PBSt muscles of 5 cats were investigated with regard to fusimotor reflex effects evoked by activity in ipsi- and contralateral hind limb muscle, skin and joint afferents (see METHODS). It was shown that the method permits simultaneous recordings of up to 4 single spindle afferents. Although only a few sets (i.e. 3 pairs, one triplet and one quadruplet) of afferents were investigated, the results indicated that the fusimotor reflex effects evoked on different spindle afferents in a given situation may vary considerably. With regard to the individual spindle afferents, a great amount of variability, both in type (excitatory and inhibitory, static and dynamic) and size of the evoked fusimotor effects, was found during different types of reflex stimulations.

The reflex nature of the effects. During these experiments, and for both muscles, the occurrence of skeletomotor activity and/or mechanical interactions were detected by recording the muscle force signals (recorded via the pullers: lowermost detection limit = 0.01 N). Neither increased force signals from the GS and PBSt muscles nor EMG activity were observed during the stimulations. Therefore, the reflex effects observed are tentatively ascribed to activity in γ-motoneurones rather than in β- or α-motoneurones.

Papers IV and VI

The aim of these studies was to investigate, using the multi-afferent recording technique described in Paper III, whether activation of receptor afferents in the PCL may evoke reflex effects on fusimotor neurones, which are potent enough to cause changes in the responses of muscle spindle afferents from the PBSt and the GS muscles. Altogether, 48 muscle spindle afferents (29 primary and 1 secondary from the GS muscle, and 17 primary and 1 secondary from the PBSt muscles) were investigated in 10 cats. The main finding was that a high proportion of the primary spindle afferents (i.e. 54%, 17 from GS and 8 from PBSt) were significantly influenced by increased tension of the PCL. Small but significant effects were also recorded from the two secondary afferents. Fig. 4 shows the type and size of the reflex effects for all the 27 afferents which exhibited statistically significant alterations (at 5% level, paired t-test) in depth of modulation and/or in fitted mean of the sinusoidal response. Each unit is illustrated by a single symbol (squares, PBSt units; triangles, GS units) representing the mean values of a number (5-12) of consecutive control-test responses (each control and
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Fig. 4. Scatter diagram showing the average change in sinusoidal response of 18 GS and 9 PBSt muscle spindle afferents for which statistically significant reflex effects were evoked by stretch of the PCL. For each afferent unit the average change in modulation (triangles: GS afferents; squares: PBSt afferents) is plotted against the average change in fitted mean. Symbols representing afferents which were investigated simultaneously are connected with lines. The 2 reflexly responsive secondary muscle spindle afferents are marked by arrows. Same reference lines as in Fig. 2. The mean values of changes in sinusoidal response were, for the primary spindle afferents from the GS muscle (n=17): 6.1 impulses/s ± 7.2 (S.D.) (fitted mean), and -1.5 impulses/s ± 6.1 (modulation). The mean values of changes in sinusoidal response were, for the primary spindle afferents from the PBSt muscles (n=8): 10.4 impulses/s ± 6.9 (fitted mean), and -4.9 impulses/s ± 4.5 (modulation).

Fusimotor reflex effects evoked on PBSt and GS spindle afferents. In Fig. 4 it can be seen that stretching of the PCL evoked mainly excitatory reflex effects on both GS and PBSt fusimotor neurones. However, for a few afferents, inhibition (symbols in the left quadrants of the diagram) of the fusimotor drive seems to have occurred and for other afferents, excitatory fusimotor effects seem to be combined with inhibitory effects (i.e. blends of excitatory dynamic and inhibitory static, or mixtures of inhibitory dynamic and excitatory static effects). Furthermore, the GS afferents showed dynamic, static as well as mixed fusimotor reflex effects, while the PBSt afferents exhibited only static and mixed reflex effects.

Reflex effects simultaneously evoked on different spindle afferents. The sets of simultaneously recorded afferents (units connected with lines), in which at least 2 afferents exhibited significant reflex effects, revealed a considerable degree of variability in the type as well as in the size of the reflex effects evoked. Thus, only in 3 sets, 2 or more afferents showed reflex actions with the same balance between the dynamic and static components of the response. In this context it should be remarked that all units in Fig. 4 were recorded in sets together with other units. Thus, all the units which are not connected with lines were recorded in sets where the other units did not respond to the PCL stretch.
A number of the spindle afferents of this study were included in more than one set and were thus tested with identical PCL stretch on more than one occasion. For some of these afferents the induced reflex effects were found to change spontaneously from one test occasion to another. The most common change was from effect to no effect or vice versa, but for a few afferents a change in the character (e.g. from dynamic to mixed or static reflexes) of the effects was obtained (see Fig. 5 in Paper VI).

**Relationship between the size of the load and the reflex effects.** The standard load used to stretch the PCL was 5-40 N. On occasions, gradually increased loads ranging from 5 to 40 N were used in attempts to assess the dependence between the size of the fusimotor effect evoked and the size of the load applied to the PCL. For a primary spindle afferent from the PBSt muscles, the results from such an attempt is shown in Fig. 5. In this figure the mean change in modulation and in fitted mean (i.e. mean values for a number of consecutive control-test responses; each control and test response being the result of 10 averaged sinusoidal stretching cycles) are shown for each traction force tested. It can be seen that, for this afferent, the threshold load for appearance of the fusimotor effect is to be found between 5 and 10 N and that the size of the reflex effect grew further when the load was increased up to 40 N.

**The reflex nature of the effects.** It was evidenced by a number of observations that the changes in the responses of the muscle spindle afferents, induced by stretch of the PCL, were caused by spinal reflexes due to activity in stretch activated receptor af-
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Fig. 6. Scatter diagram showing the average change in sinusoidal response of 35 GS (33 primary and 2 secondary) and 16 PBSt (10 primary and 6 secondary) muscle spindle afferents for which statistically significant reflex effects were evoked by stretch of the ACL. Primary spindle afferents from the GS and the PBSt muscles are displayed separately in A and B, respectively. Secondary spindle afferents from both muscles are plotted together in C. For each afferent unit, the average change in modulation (triangles: GS afferents; squares: PBSt afferents) is plotted against the average change in fitted mean. The mean values of changes in sinusoidal response were, for the primary spindle afferents from the GS muscle (in A): 1.6 impulses/s ± 2.4 (S.D.) (fitted mean), and 2.0 impulses/s ± 3.1 (modulation). The mean values of changes in sinusoidal response were, for the primary spindle afferents from the PBSt muscles (in B): 0.0 impulses/s ± 3.4 (fitted mean), and -0.5 impulses/s ± 4.1 (modulation). The mean values of changes in sinusoidal response were, for the secondary spindle afferents from the GS muscle (in C): 0.4 impulses/s ± 0.2 (fitted mean), and 0.4 impulses/s ± 0.4 (modulation). The mean values of changes in sinusoidal response were, for the secondary spindle afferents from the PBSt muscles (in C): 3.8 impulses/s ± 5.0 (fitted mean), and -1.2 impulses/s ± 0.8 (modulation). The same reference lines as in Fig. 2, 4 and 5.

In this report, fusimotor reflex effects evoked by increased tension in the ACL were investigated on 63 primary (45 from GS, and 18 from PBSt muscles) and 15 secondary (5 from GS and 10 from PBSt muscles) spindle afferents in 11 cats. It was found that activation of ACL receptor afferents elicited clear-cut changes in the sinusoidal responses of both primary and secondary spindle afferents, from both the GS muscle and the PBSt muscles. The scatter diagrams of Fig. 6 show the type and size of the reflex effects evoked on the spindle afferents, for which statistically significant (paired t-test, $P < 0.05$) alterations in fitted mean and/or in depth of modulation were encountered. Each afferent unit is illustrated by a single symbol (squares,
PBSt units: triangles, GS units) that shows the mean values in fitted mean and in modulation for a number (5-11) of control test responses (each control and test response being the result of 10 averaged sinusoidal stretching cycles).

Fusimotor reflex effects evoked on primary spindle afferents. Fig. 6 (A and B) reveals that increased tension in the ACL elicited excitatory as well as inhibitory fusimotor reflex effects in both PBSt and GS primary spindle afferents. Excitatory and inhibitory effects occurred with almost equal frequency among the PBSt primaries, while excitatory effects predominated for the GS primaries. Moreover, for the majority of primary spindle afferents, from both muscles, the induced changes in the sinusoidal responses seem to have been caused mainly by reflexes on dynamic fusimotor neurones. The difference between GS and PBSt afferents was statistically significant (grouped Mann-Whitney test; \( P < 0.05 \)) with respect to the average changes both in fitted mean and in modulation. Yet, caution in the interpretation of the difference is indicated since 4 of the 5 in the left hand part of the diagram B (all except one of the two lowermost units) were simultaneously recorded, and therefore it can not be excluded that a setting of the spinal interneuronal network, favourable to inhibition of PBSt fusimotor neurones, may have introduced a bias in the sample.

Fusimotor reflex effects evoked on secondary spindle afferents. Eight out of the 15 investigated secondary spindle afferents exhibited significant fusimotor reflex effects on activation of receptor afferents in the ACL (Fig. 6C). Both excitatory and inhibitory fusimotor reflex effects were obtained. The size of the evoked reflex effects were surprisingly large for 4 of the secondaries (all from PBSt), while only small changes in fitted mean and/or in modulation were elicited on the other secondary spindle afferents (2 from GS and 2 from PBSt).

Simultaneously evoked fusimotor reflex effects. In this investigation, as in Papers III, IV and VI, recordings were made simultaneously from sets of single spindle afferents. In general, a considerable amount of variability, with regard to the type and the size of the fusimotor reflex effects, evoked by stimulation of the ACL, were observed on the simultaneously recorded spindle afferents.

Relationship between the size of the load and the reflex effects. All afferent units of the present study were investigated by stretching the ACL with 40 N. However, during the investigation of some afferents, loads ranging from 5 to 70 N were used in order to determine the threshold load for appearance of the fusimotor reflex effects, and to evaluate the relationship between the type and size of the fusimotor effects and the degree of stretch applied to the ACL. It was regularly observed that significant fusimotor reflex effects appeared at loads just below 30 N, and that the evoked effects increased in size when the ligament was further stretched. On one occasion (for a PBSt afferent) the type of the evoked reflex effect was also found to change when the traction force was increased. This afferent showed inhibition of both static and dynamic fusimotor neurones at 30-40 N, but inhibition predominantly of dynamic fusimotor neurones at 70 N (see Fig. 5 in Paper VII).

The reflex nature of the effects. There are three lines of evidence indicating that the changes in the responses of the spindle afferents induced by stretch of the ACL were caused by fusimotor reflexes and not by some accidental mechanical coupling between the ACL and the receptor bearing muscles (GS and PBSt). First, the ipsilateral hind limb was always firmly immobilized, by pins inserted into the bones of the pelvis,
femur and tibia (cf. METHODS), and it was regularly ensured that the stretching of the ligament did not cause any visible movements of the receptor bearing muscles. Secondly, most units investigated showed a considerable response variability in spite of identical stretch of the ACL. Thirdly, deepening of the general anaesthesia either abolished or greatly decreased the effects, and the effects could also disappear spontaneously. Furthermore, for two thirds of the units, no increased force signals from the GS and PBSt muscles (recorded via the pullers: lowermost detection limit = 0.01 N) were observed during the stimuli. Small force changes were observed in 4 of 11 experiments, but the units recorded during these experiments did not stand out from the rest of the population. Therefore, the effects observed are tentatively ascribed to reflexes on γ-motoneurones rather than on β- or α-motoneurones.

Paper V

This morphological study was undertaken in order to investigate the occurrence of different types of sensory endings the cat cruciate ligaments. The cruciate ligaments (4 PCL and 3 ACL from two cats) were prepared together with the bones of the ligament insertion, stained with gold chloride, frozen, and sectioned longitudinally on a freeze-microtome. The light microscope examination of the serial sections revealed that the ACL as well as the PCL contained 4 different types of nerve endings: (1) Ruffini endings, (2) Pacinian corpuscles, (3) Golgi tendon organ-like endings, and (4) free nerve endings. Although no quantification of the sensory endings was made, it seemed that Ruffini endings and Pacinian corpuscles occurred most frequently. Moreover, the sensory endings were most densely distributed just beneath the external synovial membrane of the ligaments or close to the tibial and the femoral insertions. The frequency of occurrence and the location of the sensory endings were very similar for the ACL and the PCL.
DISCUSSION

The results presented in this thesis clearly show that reflex actions of joint afferents on both static and dynamic γ-motoneurones are strong enough to significantly influence the activity of muscle spindle afferents. Mechanical stimulation of the ipsi- and contralateral knee and ankle joint capsules were frequently found to evoke potent reflex effects on spindle afferent responses from the GS muscle. Moderately increased tension of the cruciate ligaments was also shown to regularly and potently change the activity of primary as well as secondary muscle spindle afferents from the GS and PBSt muscles. The findings indicate that the cruciate ligaments, and probably the capsules as well, may play an important sensory role. It is concluded that receptors in the cruciate ligaments and the joint capsules may contribute, via reflex actions on the γ-muscle-spindle system, to the regulation of muscular stiffness around the knee joint and thereby to the control of joint stiffness and joint stability.

Did joint receptor afferents cause the effects observed?

Electrical stimulation of the PAN. For electrical stimulation of joint afferents the PAN was used (Paper I and II). This joint nerve is the largest of the three main knee joint nerves (cf. INTRODUCTION- Innervation of the knee joint) and is sufficiently constant to permit ready identification and preparation. The PAN is also known to innervate various articular structures, i.e. the posterior aspect of the capsule, the posterior fat pads, the posterior oblique ligament, the medial and lateral collateral ligaments, the posterior cruciate ligament, and the posterior parts of the annular ligaments surrounding the lateral and medial menisci (Gardner 1944; Skoglund 1956; Freeman & Wyke 1967a). It has also been reported that in about every second animal, muscle spindle afferents from the popliteus muscle may take their course in the PAN (McIntyre et al. 1978a). Thus, the contribution from muscular receptor afferents to some of the observed reflex effects cannot be entirely excluded. Yet, it should be pointed out in this context, that reflex effects induced by stimulation of the PAN were encountered in almost all animals in the present investigation.

Natural stimulation of joint afferents. In order to activate articular sensory endings, different types of physiological stimulation were employed. These stimuli comprised both rather nonspecific types (passive joint extension and flexion, in Paper II), and more specific stimulations of defined joint structures (capsules, in Papers II and III; cruciate ligaments, in Papers IV, VI and VII). Most likely, these reflex stimuli caused activation of joint receptor afferents, but they may also to some degree have influenced cutaneous and muscle receptors in the surrounding tissues. It is also conceivable that minor direct mechanical interactions between the stimulus and the receptor bearing muscles sometimes might have occurred.

However, several lines of evidence strongly indicate that the changes in spindle afferent activity were due to spinal fusimotor reflexes, which were evoked by stimulation of articular sensory endings:

- First, the ipsilateral hind limb was always firmly fixed by metal pins inserted into the bones of the pelvis, femur and tibia. Except in the experiments where flexion/ex-
tension movements of the contralateral hind limb were used as stimuli, this limb was also fixed to the metal frame in a similar way as the ipsilateral one. By these fixations, it was ensured that the joint stimulations did not cause any discernible movement of tibia, femur, receptor bearing muscles, or surrounding tissues.

- Secondly, most spindle afferents showed a considerable response variability despite identical stimulations, indicating variations of the spontaneous fusimotor drive.

- Thirdly, the observed effects could also disappear spontaneously or as a result of deterioration of the spinal circulation.

- Fourthly, the reflex nature of the effects was further supported by the findings that deepening of the general chloralose anaesthesia either wiped out the effects or reduced them considerably.

- Fifthly, possible activation of skin and muscle receptor afferents was always largely eliminated by denervations of surrounding structures and in some cases also by tenotomies and/or decutanizations (Paper II).

- Sixthly, fusimotor reflexes evoked by the physiological stimuli were effectively abolished by cutting off relevant joint nerves (Papers II, IV and VI), or by intra-articular injections of local anaesthetics (Paper II). The latter, "functional denervation" induced by local anaesthesia, has been demonstrated to efficiently silence intra-capsular sensory nerve fibres (e.g. Clark et al. 1979; Ferrell 1980; Baxendale & Ferrell 1985; Ferrell et al. 1985).

- Finally, during the experiments in which stretches of the cruciate ligaments were used as stimuli, the occurrence of skeletomotor activity and/or mechanical interactions were detected by recording the muscle force signals via the pullers. Since the recording of the force signals was very sensitive (lowermost detection limit = 0.01 N) it seems unlikely that mechanical interactions of any significance between the stimuli and the receptor bearing muscles would have escaped detection. For most of the units no increased force signals were observed during the stimulations, and the units, for which small force changes could be observed, did not stand out from the rest of the populations.

The reflexly induced changes in muscle spindle afferent activities were obtained both in spinalized and in non-spinalized preparations and were usually not correlated to efferal muscle activity, as determined by observing the EMG activity or the force development of the receptor bearing muscles. Therefore, the reflex effects observed are tentatively ascribed to activity in \( \gamma \)-motoneurones rather than in \( \beta \)- or \( \alpha \)-motoneurones.

Receptor categories accountable for the reflex effects

**Electrical stimulation of the PAN.** In the studies in which knee joint receptor afferents were activated by graded electrical stimulation of the PAN (Papers I and II), some \( \gamma \)-cells were found to be influenced only from low threshold afferents, some only from high threshold afferents and some from both low and high threshold afferents (see below: *Responses to electrical stimulation of joint afferents*). May the effects elicited at different intensities be related to stimulation of fibres from different types of sensory endings? According to a number of investigations (Skoglund 1956;
Freeman & Wyke 1967a; Boyd & Davey 1968; Burgess & Clark 1969; Ferrell 1980; Heppelmann et al. 1988) it seems that the afferents with lowest thresholds to electrical stimulation are fibres from Golgi tendon organ-like endings and Pacinian corpuscles, and that the fibres from Ruffini endings and free nerve endings have higher thresholds. It should, however, be remembered that there is a considerable overlap between afferents from different receptor types with regard to fibre diameter, conduction velocity and threshold, and also that the PAN may contain some popliteus muscle afferents (see above: Did joint receptor afferents cause the effects observed?).

**Natural stimulation of joint afferents.** The occurrence of various types of sensory nerve endings in knee joint tissues has been reported in many papers (for references, see INTRODUCTION - Sensory endings in the knee joint). Thus, in man, Golgi tendon organ-like endings, Ruffini endings, Pacinian corpuscles and free nerve endings have been found in the knee joint capsule, ligaments and menisci. Different types of nerve endings have also been identified in the cat knee joint. Ruffini endings, Pacinian corpuscles and free nerve endings have been demonstrated in the cat capsule and meniscus, and Golgi tendon organ-like endings, Ruffini endings and free nerve endings in the collateral ligaments. Until the morphological investigation presented in Paper V, the cat cruciate ligaments were believed to accommodate only Golgi tendon organ-like endings and free nerve endings. However, from Paper V it is clear that the cruciate ligaments of the cat also contain Ruffini endings and Pacinian corpuscles. Thus, it seems that the cat and the human the cruciate ligaments are endowed with the same principal types of nerve endings, which, in this respect, makes the cat a suitable animal for studies of the sensory function and patophysiology of the cruciate ligaments.

Neurophysiological studies have revealed that the behavioural characteristics are different for disparate morphological types of sensory endings (for references, see INTRODUCTION - Sensory endings in the knee joint). Ruffini endings and Pacinian corpuscles are known to have low thresholds to mechanical deformation, while Golgi tendon organ-like endings and free nerve endings have higher thresholds. Furthermore, Pacinian corpuscles are rapidly adapting, while Ruffini and Golgi tendon organ-like endings are slowly adapting mechanoreceptors.

The physiological stimuli used in the present series of investigations (i.e. pressure applied to joint capsules, flexion/extension movements of joints and increased tension of the cruciate ligaments) were always applied before, and maintained during, the data collections. Since tonic stimuli were used and since the effects nearly always lasted as long as, but ceased after termination of, the stimuli, activity in slowly adapting sensory endings seems to be the most likely cause for the evoked reflex effects. Considering also that the fusimotor reflex effects were always obtained with mechanical stimulations which were kept within the nonnoxious range, it seems that the most likely candidates to give the effects accounted for in these reports are Ruffini endings and/or perhaps Golgi tendon organ-like endings.

The standard stimulation of the cruciate ligaments used in the present investigation (Paper IV, VI and VII), consisting of a tonically applied low (5-40 N) traction force, indicates that most of the observed effects on the spindle afferent responses were caused by slowly adapting receptors with low thresholds to mechanical deformation. Since the actual threshold loads necessary to elicit reflex actions were only tested on a few occasions, it cannot be excluded that the threshold loads sometimes might...
be lower than those found in this investigation (5-10 N for PCL and 30 N for ACL). Yet, a load of 5-40 N must probably be regarded as moderate since 210-300 N might be used without ligament rupture (cf. also Solomonow et al. 1987). The considerable "safety margin" between the thresholds and the forces needed to tear the ligaments strongly indicates that the reflexes are not of nociceptive character. It is worth noting that during normal activity (not strenuous) the human ACL is supposed to be subjected to traction forces amounting to about 25% of those necessary to tear the ligament (Noyes et al. 1984).

Responses to electrical stimulation of joint afferents

Responsiveness. Possibly the most important result obtained in the experiments, in which post-synaptic potentials were recorded with micro-electrodes from γ-cells (Paper I), was the high general responsiveness to electrical stimulation of the PAN, demonstrated both for static and for dynamic γ-motoneurones (93% of the classified γ-cells were responsive). A comparison of the responsiveness found, for the same population of cells, during stimulation of nerves from other hind limb receptor categories, reveals that the responsiveness to PAN stimulation was of the same magnitude as that found with stimulation of different (sural, peroneal and tibial nerves) ipsilateral cutaneous nerves (95% responsive γ-cells; Johansson & Sojka 1985), and slightly larger than that obtained for activation of group II and III muscle afferents from various ipsilateral muscle nerves (86% responsive γ-cells; Appelberg et al. 1983b,c). Thus, the divergence from this rather thin nerve (which contains relatively few fibres compared to the muscle and cutaneous nerves used for stimulation), in the pathways to fusimotor neurones projecting to different hind limb muscles, appears to be surprisingly large.

Thresholds. High threshold excitatory and inhibitory effects in response to electrical stimulation of the PAN were demonstrated already in the early investigations of joint reflexes on γ-motoneurones, by recordings from γ-cells or γ-efferents (Voorhoeve & van Kanten 1962; Grillner et al. 1969). In 1978, McIntyre and coworkers (1978b), in a study in which indirect evaluation of fusimotor activity was made by recording from primary muscle spindle afferents, found fusimotor reflex effects only at stimulation strengths that exceeded 2 times the threshold for the most excitable PAN afferents. These observations contrast with the results obtained from γ-cells (Paper I) as well as with those obtained from muscle spindle afferents (Paper II), which showed clear-cut reflex effects at stimulation strengths as low as 1.1-1.5 times the threshold for evoking of a compound action potential in the PAN. It is also shown in Paper I and II that excitatory as well as inhibitory reflex effects (on flexor and extensor γ-cells of both static and dynamic type) can be evoked at both low and high stimulation strengths. In addition, with regard to the occurrence of low and high threshold excitatory and inhibitory effects, a considerable heterogeneity was found among all subpopulations of fusimotor neurones (i.e. flexor and extensor γ-cells of both static and dynamic type).

General pattern of effects. From the results reported in Papers I and II it is clear that electrical stimulation of the PAN can evoke both excitatory and inhibitory effects in flexor and extensor fusimotor neurones of both the static and the dynamic type. These results conform to those found by Grillner et al. (1969), but disagree with the observations made by McIntyre et al. (1978b). The latter authors found only excitatory fusimotor effects on the spindle afferents investigated, and concluded
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on indirect evidence that these effects were primarily mediated via static fusimotor neurones.

**Synaptic coupling.** The calculations of the segmental delays were partly based on the assumption that the effects were transmitted via the most rapidly conducting PAN fibres (assumed conduction velocity = 90 m/s, cf. Boyd & Davey 1968; Burgess & Clark 1969; Ferrell 1980). Although this assumption probably leads to a small overestimation of the figures for the shortest segmental delays, it seems likely that the fastest excitatory pathway to dynamic γ-cells is trisynaptic (cf. Lundberg *et al.* 1978), or perhaps even disynaptic (cf. Harrison & Jankowska 1985), whereas the fastest excitatory pathway to static γ-motoneurones and the shortest inhibitory route to both types of cells probably includes at least one synapse more.

**Responses to physiological stimulation of joint afferents**

*Responsiveness.* The most prominent finding obtained in Papers II, III, IV, VI and VII was that physiologically induced activity in ipsi- as well as contralateral joint receptor afferents frequently exerts potent reflex actions on fusimotor neurones, which are strong enough to significantly alter the discharge of the muscle spindle afferents. A considerable responsiveness was encountered for all different types of mechanical stimuli. Thus, 23% of the GS primary spindle afferents studied with pressure on the contralateral knee-joint capsule (Paper II), and 28% of the GS primary spindle afferents studied with pressure applied to the ipsilateral knee joint capsule (Paper II), showed significant fusimotor reflex effects. Increased tension in the cruciate ligaments was found to be even more potent in evoking reflex effects on spindle afferents (Papers IV, VI and VII). Out of the total samples of GS and PBSt primary spindle afferents, increased tension in the PCL caused significant reflex effects on 54% (59% of the GS primaries and 47% of the PBSt primaries), and stimulation of the ACL on as much as 68% of the units (73% of the GS primaries and 55% of the PBSt primaries). A high general responsiveness (41%) to natural non-noxious stimulation of the ipsilateral knee joint, obtained by recording from γ-efferents, was recently also reported by He *et al.* (1988). In the light of these results it may be concluded that the activity in knee joint receptor afferents, both from the capsule and cruciate ligaments, are significantly involved in the reflex control of the γ-muscle-spindle systems of hind limb muscles. The results also seem to indicate that the cruciate ligaments may play a more important sensory role than hitherto believed.

Although the relative number of spindle afferents responsive to natural stimulation of joint receptors afferents appears to be quite high, it might be argued that the responsiveness among the γ-cells to electrical stimulation of the PAN was significantly higher. However, since electrical stimulation causes a higher degree of synchrony and convergence, it comes as no surprise that physiological stimulation of joint afferents yielded a smaller general responsiveness figure.

**General pattern of effects.** Both ipsi- and contralateral knee joint receptor stimulations were found to evoke excitatory as well as inhibitory fusimotor reflex effects. A majority of the GS spindle afferents, which were responsive to increased tension of the cruciate ligaments (i.e. both PCL and ACL), showed excitatory effects. All the PBSt spindle afferents exhibited excitatory fusimotor effects in response to stretching of the PCL, while increased tension in the ACL elicited an approximately equal frequency of excitatory and inhibitory responses among the PBSt afferents (cf. RESULTS—Paper VII). It is interesting to note that inhibitory fusimotor effects were more frequently encountered for ipsilateral capsule stimulation. Yet, the overall impression is
that physiological stimulation of joint receptor afferents in the hind limbs predominantly give excitatory fusimotor reflex effects (cf. He et al. 1988).

With recordings from spindle afferents, it is quite likely that the number of inhibitory effects are somewhat underestimated. This is due to the fact that some degree of spontaneous activity is necessary for the detection of purely inhibitory effects. Furthermore, in semi-spinalized chloralose anaesthetized cats most of the dynamic \( \gamma \)-motoneurones have been found to be spontaneously active, whilst that seems to be the case only for about 30\% of the static \( \gamma \)-motoneurones (Appelberg et al. 1982a). Thus, it is possible that the number of inhibitory static effects are slightly more underestimated than the number of inhibitory dynamic effects.

The natural stimulations of joint afferents used in the present series of experiments were shown to evoke different types of reflex effects, including pure static, pure dynamic as well as mixed fusimotor effects with different amounts of static and dynamic influences. For all types of reflex stimuli, except for ipsilateral knee joint pressure, for which static, dynamic and mixed effects occurred about equally frequently, a majority of the GS primary spindle afferents exhibited pure or predominantly dynamic fusimotor effects. This was probably also the case for most of the primary spindle afferents from the PBSt muscles during stretching of the ACL. When the PCL was stretched, on the other hand, pure static or mixed fusimotor reflex effects seemed to predominate on the PBSt primary afferents. Thus, the results show that sensory endings in both the capsules and the cruciate ligaments can evoke reflex effects on dynamic and static fusimotor neurones, which are sufficiently strong to significantly alter the sensitivity of the muscle spindle afferents.

**Variability in reflex effects evoked on the individual \( \gamma \)-cells and spindle afferents**

In a series of investigations from our laboratory, in which recordings were made from single \( \gamma \)-cells (Paper I; Appelberg et al. 1982a; 1983a,b,c,d; Johansson 1981, 1988; Johansson & Sojka 1985; Sojka 1985), it was found that hind limb \( \gamma \)-motoneurones have very complex and individualized receptive profiles with respect to the pattern of reflex effects evoked by electrical stimulation of descending pathways and ipsilateral hind limb nerves. The individualized receptive profiles of the \( \gamma \)-motoneurones are also reflected at the level of the muscle spindle afferents. This has been demonstrated using natural stimulation of muscle, skin and joint receptor afferents in the ipsi- as well as in the contralateral hind limbs (Papers II, III, IV, VI and VII; Appelberg et al. 1982b, 1984, 1986; Johansson 1981; Sojka 1985; Johansson et al. 1989). Based on such observations, a complementary view on the role of the \( \gamma \)-muscle-spindle system was proposed, i.e. the final common input hypothesis (Johansson 1981; Appelberg et al. 1983c). This hypothesis implies that information mediated by the muscle spindle afferents is shaped, not only by variations in muscle length, but to a large extent also by the signals from descending pathways and from ipsi- and contralateral peripheral nerves (i.e. from muscles, skin and joints). Accordingly, it was suggested that descending messages and peripheral receptor information are integrated in the fusimotor neurones and then transmitted to the muscle spindles, where this integrated information undergoes final adjustments according to the ongoing length/tension changes of the parent muscle. In this way, the \( \gamma \)-spindle system is proposed to constitute a premotoneuronal integrative system, which conveys polymodal feedback to the CNS. It seems that the intricate reflex regulation of the \( \gamma \)-spindle system may, in situations with a high gain in the \( \gamma \)-spindle loop, be a mechanism well suited to deal with the sophisticated coordination between different muscles and perhaps also between various intramuscular compartments (Johansson 1985; Johansson & Sojka 1985). It has also been
hypothesized (Johansson & Sojka 1985; Paper III) that the individualized and polymodal responses of the primary muscle spindle afferents throw some light on the mechanism by which these afferents are capable of providing the CNS with information about the positions and movements of the limbs (cf. Goodwin et al. 1972). Since the different primary muscle spindle afferents transmit differently composed multisensory information, adjusted in the spindles according to the ongoing movements, to the CNS it seems possible that the CNS might extract the complex picture of limb positions and movements from a number of spindle afferents (Johansson 1985).

The results of the present study demonstrate that, behind the general features discussed in the previous sections, there was a considerable amount of variation concealed in the fusimotor reflex effects evoked during stimulation of joint receptor afferents. It can therefore be concluded that the polymodal and individualized messages transmitted in the spindle afferents also contain information from ipsi- and contralateral joint afferents. An important finding in this context, was the high degree of variability which was observed on simultaneously investigated spindle afferents. Since these afferents were recorded during identical conditions, as concerns descending activity and 'setting' of the spinal interneuronal network, it seems most likely that the observed diversity in fusimotor reflex effects, as is postulated in the "final common input" hypothesis, reflects a basic property of the organization of the fusimotor reflex control.

It should be recalled that the 'setting' of the spinal interneuronal network and/or the descending drive may vary considerably between different preparations, from one experiment to another, and even during one and the same experiment (see Fig. 5 in Paper VI; cf. also Loeb & Duysens 1979; Prochazka & Hulliger 1983). The diversity in fusimotor responses observed by investigating one single γ-cell/spindle afferent at a time may therefore to some extent be the result of spontaneous fluctuations in spinal or descending conditions, which may occur during the time intervals between the recordings.

In the experiments, in which reflex effects from the cruciate ligaments were investigated (Papers IV, VI and VII), another finding was made that might be relevant in this context. Namely, that the fusimotor reflex effects evoked on a given set of spindle afferents sometimes spontaneously changed character (i.e. changes in both type and size) during the course of the experiment (see Fig. 5 in Paper VI). Thus, for instance, a first control-test series might reveal both static and dynamic reflex actions, while on a second test occasion, made on the same afferents with identically performed reflex stimulation, exclusively static fusimotor effects might be encountered. These findings were probably due to spontaneous changes in the 'setting' of the spinal reflex network, and they seem to indicate that the response profiles are a variable phenomenon and that the reflex pathways to the fusimotor system comprise a number of alternatives. The wide and individualized response profiles of the fusimotor neurones, together with the existence of different optional fusimotor reflex pathways, might provide a basis for the command centers in their tailoring of fusimotor programmes (cf. Loeb et al. 1985). It is conceivable that certain groups of fusimotor neurones, with specific receptive fields, are 'selected' in order to provide appropriate sensory feedback for the accurate execution of different motor tasks (cf. Loeb 1984).

The complexity of the γ-muscle-spindle system observed in studies at our laboratory seems to be in good accordance with a number of recent findings from other laboratories. Thus, Schwestka et al. (1981) observed that fusimotor activity induced complex actions on the correlation between primary spindle afferent discharge patterns, so that some spindle afferents were decorrelated while other new correlations appeared (cf. also Inbar
et al. 1979). Moreover, the research group in Göttingen (Windhorst et al. 1976; Windhorst & Meyer-Lohmann 1977) has suggested that ‘the total "sensory space" of a muscle can be divided into overlapping "sub-spaces"’ which are likely to require ‘specified, motor innervation patterns, which might, at least in part, be established or influenced by the specific feedback signals originating from the respective compartment’. Based on studies of the anatomical arrangement of muscle spindles and motor units within a muscle, a similar idea has been proposed by Binder & Stuart (1980). These authors suggested that the muscle spindles ‘generate a sensory partitioning of the motor-unit population within the muscle’.

Comparison with joint reflex effects evoked on skeletomotoneurones

Several findings indicate that the activity in low threshold mechanoreceptor afferents from the knee joint primarily influences the γ-spindle system, rather than causes reflex effects ‘directly’ on the α-motoneurones. Firstly, a comparison of the responsiveness for γ and α-motoneurones to low threshold electrical stimulation of the PAN (below 2 x PAN threshold), reveals that γ-motoneurones are much more frequently influenced at these stimulation intensities (Paper I; Lundberg et al. 1978; see also Eccles & Lundberg 1959). Secondly, in a recent study, He et al. (1988) investigated reflex effects on γ and α-efferents to mechanical stimulation of knee joint receptor afferents before and after introduction of intra-articular knee joint inflammation. It was reported that under the control conditions (i.e. before inflammation) only 14% of the α-motoneurones showed a response to mechanical stimulation, whereas 41% of the γ-motoneurones were responsive. Thirdly, the fusimotor reflex effects evoked by the mechanical stimulations used in the present investigations (Papers II, III, IV, VI and VII), were, with a few exceptions, not correlated to extrafusal muscle activity (see above: Did joint receptor afferents cause the effects observed?). This finding is consistent with that reported by Solomonow et al. (1987), who stated that large loads (around 130 N) had to be applied to the ACL in order to evoke reflex changes in the EMG signals from the hamstrings and quadriceps muscles. It should be recalled that in the present study (Paper IV, VI and VII), only moderate loads (5-40 N) were applied to the ACL during reflex stimulation.

It is at present well known that α- and γ-motoneurones are differently influenced by a number of descending and reflex inputs (for reviews, see Baldissera et al. 1981; Hulliger 1984; Johansson 1981, 1985, 1988), and that the ultrastructure and number of synapses on lumbar γ and α-motoneurones are different (Lagerbäck 1985; Johnson 1986). The diversity and complexity of the reflexes on the γ-muscle-spindle system contrasts with the relative rigidity of the corresponding reflexes on α-motoneurones. The reflex organization which gives the fusimotor neurones a receptive profile of their own has been suggested to be an important property for the integrative function proposed for the fusimotor-spindle system (see above: Variability in reflex effects evoked on the individual γ-cells and spindle afferents). It may also be inferred that it appears unlikely that the rather stereotype reflexes to α-motoneurones convey the complex type of information needed for coordination of finely regulated movements (Johansson 1981). Accordingly, it is conceivable that the delicate receptor messages required for reflex adjustment of the programmes for finely tuned movements (or phases of movements) are gated by and integrated in the fusimotor system, while the cruder type of information are transmitted directly to the skeletomotor neurones (cf. Johansson 1981).
A mechanism through which joint receptor afferents may regulate joint stability

A number of authors have hypothesized that articular sensory endings may function by initiating joint protective reflexes to the muscles around the joint (e.g. Abbott et al. 1944; Palmer 1958; Stener 1959; Burgess & Clark 1969; Lundberg et al. 1978; Grigg 1975; Kennedy et al. 1982; Solomonow et al. 1987; Zimny 1988a). However, the functional significance of such reflexes has been seriously questioned by Pope et al. (1979) (cf. also Rack 1981). They concluded that such reflexes are too slow to be able to protect the joints from injuries.

Other authors have advocated the view that impulses arising from joint sensory endings have a role in maintaining a normal, smooth, coordination of the muscle tone in different movements and in posture (e.g. Abbott et al. 1944; Freeman & Wyke 1967b; duToit 1987). In 1967, Freeman & Wyke suggested, on indirect evidence, that the control of muscle tone was achieved by continuous actions on the γ-motoneurones, via polysynaptic reflexes from articular receptors (Freeman & Wyke 1967b). The results accounted for in the present thesis, clearly demonstrate that natural activation of joint receptor afferents exert reflex effects on the γ-muscle-spindle system. Thus the findings seem to support the idea proposed by Freeman & Wyke. Furthermore, since the reflexes on primary spindle afferents from different muscles acting at the joint were both frequent and potent, it seems not unlikely that such a reflex control may be of large importance in the continuous regulation of muscle tone (muscle stiffness), and thereby for the joint stability (joint stiffness) provided by the muscles.

The functional stability of the knee joint is the result of a complex interplay between factors contributing to the static stability and factors subserving the dynamic stability (cf. e.g. Grimby et al. 1980; Lysholm et al. 1984). The dynamic stability of the joint is largely dependent on the load imposed on the joint and thereby also on the activity of the muscles around it (Goldfuss et al. 1973; Markolf et al. 1976, 1981; Baratta et al. 1988). It has, for instance, been demonstrated that subjects (in good physical condition), by contracting their quadriceps muscles, may increase the stiffness of their knee joints by a factor of 10 or more (Markolf et al. 1981), and it was recently suggested that co-activation of muscles is necessary to aid the ligaments in maintaining joint stability (Baratta et al. 1988).

It is well known that muscle stiffness (i.e. the ratio of force change to length change) consists of two components, (i) reflex-mediated muscle stiffness and (ii) intrinsic muscle stiffness (see e.g. Akazawa et al. 1983; Hoffer & Andreassen 1981). The intrinsic muscle stiffness is dependent on the elastic properties of the muscle and on the existing acto-myosin bonds, while the reflex-mediated stiffness is determined by the excitability in the α-motoneuronal pool. The level of activity in the motoneuron pool is dependent on descending commands and autogenic and heterogenic reflexes (Nichols 1987). It has been argued that the most important of these factors in the stiffness regulation is the autogenic reflexes provided by muscle spindle afferents (Houk et al. 1981; Allum & Mauritz 1984).

In the original theory of stiffness control it was postulated that the reflex-mediated stiffness compensates for variations in intrinsic muscle properties so as to maintain a constant total muscle stiffness over almost the entire range of muscle forces (Houk 1979; Houk & Rymer 1981; see also Hoffer & Andreassen 1981). This hypothesis was based on data obtained during static or postural conditions.
However, in several recent studies, in which muscle stiffness has been investigated during dynamic or cyclic movements, evidence has been presented for variability in the overall muscle stiffness, largely caused by modulation of the reflex-mediated component (see e.g. Aldridge et al. 1981; MacKay et al. 1980; Akazawa et al. 1983; Nichols 1987). It has been proposed by Akazawa et al. (1983) that the fluctuations in muscle stiffness during dynamic conditions may be explained by changes in muscle spindle sensitivity, due to modulation of the fusimotor activity. If this is correct, it is suggested by the results from the present investigations, that activation of joint afferents, via reflex effects on the γ-muscle-spindle system, contribute to the continuous modulation of muscle stiffness. Moreover, since sensory endings located in both the knee joint ligaments and in the capsule are known to signal, not only at extreme flexion and extension, but also at intermediate joint angles (Skoglund 1956; Burgess & Clark 1969; Ferrell 1980), it seems quite possible that these receptors, through the γ-muscle spindle system, may modify the reflex-mediated stiffness over the entire range of joint movement.

It should be recalled in this context that γ-motoneurones (for references see, Variability in reflex effects evoked on the individual γ-cells and spindle afferents), as well as spinal interneurones (see e.g. Lundberg 1979; Schaille et al. 1986, 1987b; Craig et al. 1988; Edgley & Jankowska 1987), which are influenced from joint receptors also receive input from skin and muscle receptors. Therefore, it is most likely that information from more than one receptor category is involved in the regulation of muscle stiffness.

Intrinsic muscle stiffness is thought to be dependent purely on mechanical properties of the muscle, i.e. largely on the existing acto-myosin bounds or the degree of muscle contraction at a given moment. This component of the muscle stiffness is, in other words, the result of the 'previous history' of the muscle (i.e. of the preceding reflex actions and descending commands), and it has been found to fluctuate during naturally occurring movements (Akazawa et al. 1982). According to the fact that protection of the joint by intrinsic muscle stiffness is always present, this stiffness component has been denoted "the body's first line of defense against perturbations" (Akazawa et al. 1982, 1983; see also Grillner 1972). If joint afferents significantly contribute to the regulation of the reflex-mediated muscle stiffness, then they will be of importance also for the intrinsic muscle stiffness, and thereby for the maintenance of the joint stability necessary for the protection of the joint against sudden perturbations.

Possible clinical relevance of the results

Despite the development of a number of methods for treatment of injured knee joint ligaments, the results are often somewhat disappointing for the patients. Symptoms such as functional instability, feelings of give way and muscular weakness often persist (Noyes et al. 1984). In 1965, Freeman and co-workers proposed that such symptoms might be the result of partial de-afferentation of the capsule and ligaments in association with the injured joint (Freeman et al. 1965; see also Kennedy et al. 1982; Elmqvist et al. 1988). Obviously, a de-afferentation alters the normal sensory feedback during the execution of movements. If it is assumed that different motor programmes are adapted to receive specific sensory feedback for the accurate execution of various motor tasks, it can be suggested that a ligament injury most probably causes an ensemble of sensory feedback which does not fit the existing motor programmes (cf. above: Variability in reflex effects evoked on the individual γ-cells and spindle afferents). This in turn, may induce errors in the normal coordination pattern of the muscles, and thereby also disturbances in the functional joint stability.
Experimental evidence for the participation of articular receptor afferents in maintaining the normal muscular activation pattern during posture and movements has been reported. Persistent postural and kinesthetic changes have been observed on cats after injections of local anaesthetics into the knee joint cavity (Ferrell et al. 1985), and after denervation of knee joint structures (Freeman & Wyke 1966). Recent investigations have also shown that patients with complete ACL tears have impaired limb proprioception (Barrack et al. 1989).

Considering the importance of the sensory function of the joint structures, it would seem sensible to minimize the sensory damage whenever surgical treatment is necessary. For instance, when a ruptured cruciate ligament is substituted with a graft, the sensory function of the ligament is not restored. In order to avoid a complete sensory loss, it seems reasonable to repair the injured ligament, rather than substituting it with a graft. In this context it should be mentioned that Clancy and co-workers recently reported excellent results with primary repair of the teared ACL combined with augmentation with a patellar tendon graft (Clancy et al. 1988). Considering the fact that the proximal and distal ends of the cruciate ligaments are most densely equipped with sensory endings (Paper V; Schultz et al. 1984; Zimny et al. 1986; Schutte et al. 1987), it seems important to perform the primary repair so as to avoid destruction of the ligamentous ends. In addition, it is probably crucial to re-establish a "normal tension profile" in the repaired ligament, since most receptor afferents signal within a limited range of mechanical stress (see e.g. Andrew & Dodt 1953; Boyd & Roberts 1953; Skoglund 1956; Burgess & Clark 1969; Ferrell 1980).

It is probably also important to consider the changed sensory properties of an injured joint when developing rehabilitation programmes. Thus, an injured ligament is likely to cause a persistently disturbed sensory feedback from the joint, and therefore the existing motor programmes have to be modified in accordance to the new sensory situation. In fact, the importance of sensory feedback in the process of relearning motor programmes have repeatedly been demonstrated (for reviews, see Marsden et al. 1984; Mulder & Hulstyn, 1984), and a special need of joint receptor input for the learning of the timing and positioning of the limbs has been suggested (Adams 1977). Also, balance-training programmes have been reported to improve the functional stability of sprained ankle joints (Freeman et al. 1965; Tropp 1985, 1986; Gauffin et al. 1988). Thus, as expected, the relearning process seems to be positively influenced by coordination-training, aimed at "fitting" the motor programmes to a diversified sensory influence in the new sensory situation. Accordingly, rehabilitation after knee ligament injuries should not only comprise strength training, but always be complemented with training of muscle coordination.
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REFERENCES


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combining static and dynamic fusimotor stimulation on the response of muscle spindle
primary endings to sinusoidal stretching. J. Physiol. 267, 839-856.
Grimby, G., Gustafsson, E., Peterson, L. & Renström, P. (1980). Quadriceps func-
Hagbarth, K. E. & Eklund, G. (1966). Motor effects of vibratory muscle stimuli in
man. In: Granit, R. (ed.) Muscular Afferents and Motor Control. Almqvist och Wik-
sell, Stockholm, pp. 177-186.
Halata, Z. (1977). The ultrastructure of the sensory endings in the articular capsule of
the knee joint of the domestic cat (Ruffini corpuscles and Pacinian corpuscles).
J. Anat. 124, 717-729.
Halata, Z. (1988). Ruffini corpuscles - a stretch receptor in the connective tissue of
the skin and locomotion apparatus. Prog. Brain Res. 74, 221-229.
Halata, Z. & Groth, H. R. (1976). Innervation of the synovial membrane of the
Harrison, P. J. & Jankowska, E. (1984). An intracellular study of descending and non-
cutaneous afferent input to spinocervical tract neurones in the cat. J. Physiol.
356, 245-261.
Harrison, P. J. & Jankowska, E. (1985). Sources of input to interneurones mediat-
ing group I non-reciprocal inhibition of motoneurones in the cat. J. Physiol. 361,
379-401.
the knee joint in the cat alter responses of flexor motoneurons to leg move-
and unmyelinated axons in the medial and posterior articular nerve of the cat’s knee
Hoffer, J. A. & Andreassen, S. (1981). Regulation of soleus muscle stiffness in premam-
Hofmquist, B. (1961). Crossed spinal reflex actions evoked by volleys in somatic af-
Hofmquist, B. & Lundberg, A. (1961). Differential supraspinal control of synaptic ac-
tions evoked by volleys in the flexion reflex afferents in alpha motoneurones. Acta
Physiol. Scand. 54, suppl. 186, 1-51.
41, 99-114.
in stiffness regulation - A predictive mechanism provided by non-linear feedback.
In, Muscle Receptors and Movement, eds., Taylor, A. & Prochazka, A., pp. 299-
Brooks, V. B. (ed) Handbook of Physiology, The nervous system II. American
Physiological Society, Bethesda, pp. 257-323.
Hromada, J. & Poláček, P. (1958). A contribution to the morphology of encapsu-
lated nerve endings in the joint capsule and the periarticular tissue. Acta Anat. 33,
at constant and abruptly changing rates. J. Physiol. 294, 461-482.


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