Dorsal Spinocerebellar Tract: Response Pattern of Nerve Fibers to Muscle Stretch

Abstract. *The response to muscle stretch of second-order neurons of the dorsal spinocerebellar tract was studied in anesthetized cats. Three different types of neurons are defined; the response of each type is remarkably similar to that of the corresponding stretch-receptors of the muscle. Accordingly, the cerebellum receives information on the mechanical situation in muscle from three qualitatively different channels: one has a high degree of sensitivity to dynamic stretch (Ia units) and provides a mixed signal of muscle length and speed of movement; another provides information mainly on muscle length (group-II units); and the third signals information on the degree of contraction of the muscle (Ib units).*

One must know the details of the presynaptic input in order to analyze the transfer characteristics of the successive synaptic "relays" of the various sensory pathways. Such details are known for the neurons of the dorsal spinocerebellar tract (DSCT). Lundberg *et al.* and Oscarsson (*1*) identified various functional subdivisions of the DSCT. Other workers determined the response patterns of the different types of stretch receptors in skeletal muscle to defined mechanical stimulation (*2, 3*). For studying the transfer of nervous activity from the first-order to the second-order neurons of a sensory pathway the DSCT offers the following advantages: (i) DSCT neurons are monosynaptically connected to the primary afferent fibers from the stretch receptors. (ii) Functional subdivisions of the DSCT (that is, the type of stretch receptors connected to a particular DSCT neuron) can be accurately identified. (iii) Response of the receptors to controlled physiological stimuli is constant. (iv) The stretch receptors of muscle adapt slowly. (v) The activities of single DSCT fibers can be recorded with relative ease in the dorsolateral funicles of the cord, without interference with the synaptic mechanisms in the soma-dendritic region of the neurons.

Our report concerns the activation of DSCT neurons by either extension or tetanic contraction of the gastrocnemius-soleus muscle and the anterior tibial–extensor digitorum longus (TA-EDL) muscles of the hind leg of the cat.

Sixteen cats were anesthetized intraperitoneally with Nembutal (30 mg/kg), with small additional intravenous doses when necessary. To avoid uncontrolled stimulation, all nerves in the hind leg, except those to the muscles under study, were severed. The tendons of TA-EDL muscles were tied closely together. To prevent interference from undetermined fusimotor activity, the ventral roots of L VI, L VII, and S I were divided. The muscles were stretched linearly at a rate of 19 mm/sec, and the stretch could be interrupted at any point up to the limit of physiological extension of the muscle. Tetanic contractions of the muscles were produced by stimulation of the L VII ventral root at 100 pulses per second and at intensities up to maximum for muscle contraction. Activities of single axons of the DSCT were recorded in the dorsolateral funiculus of the cord by micropipettes (filled with KCl) of 5- to 20-megohms resistance. Activity of the primary afferent fibers was recorded from thin filaments of the dorsal roots.

The DSCT neurons are connected to different types of peripheral receptors (*4*). The type most frequently encountered during microelectrode exploration of the dorsolateral funiculus had a threshold to electrical stimulation of the muscle nerve less than or equal to that of the twitch contraction of the muscle; latency to electrical stimulation was short—3 to 4 msec. During a twitch contraction of the muscle this type exhibited a characteristic pause in discharge (Fig. 1*A*). Such fibers are evidently activated from the primary receptors of the muscle spindles and are accordingly termed Ia DSCT units.

The second type of unit, which was encountered much less frequently, had a threshold above that of maximum twitch contraction; only five such units were studied systematically. Latency to electrical stimulation was 4.5 to 6 msec. During twitch contraction of the muscle there was no discharge from these units (Fig. 1*B*), suggesting that they were activated from the secondary endings of the muscle spindles. The action potentials of these (group II) DSCT units were always small and it proved difficult to record their activity for a sufficient length of time; possibly their axons were smaller.

The third type of unit (group Ib) resembled the first type both in latency and in having a threshold to electrical stimulation at or below the threshold of twitch contraction. During twitch contraction, however, group-Ib units showed a characteristic burst of activity during the twitch (Fig. 1*C*). This discharge pattern is very similar to that of the Golgi tendon organs. Besides these three main types, other fibers not described here were found in the dorsolateral funicle of the cord (*4, 5*).

Most of the Ia DSCT units showed a "spontaneous" discharge without any stretch being applied to the muscle. Of the 47 Ia units studied, only two showed no activity when the muscle was slack. The fraction of initially silent units was about the same for gastrocnemius-soleus and TA-EDL. In response to linear stretch of the muscle the Ia units displayed a characteristic discharge pattern (Fig. 2*A*, *b*). During the dynamic phase of the stretch, the frequency of firing of the unit increased progressively. At the end of the dynamic phase the mean frequency of firing decreased rapidly to a lower rate, which then decreased only slowly during a period of maintainted extension. On release, the unit immediately became inactive. All these features were regularly observed also

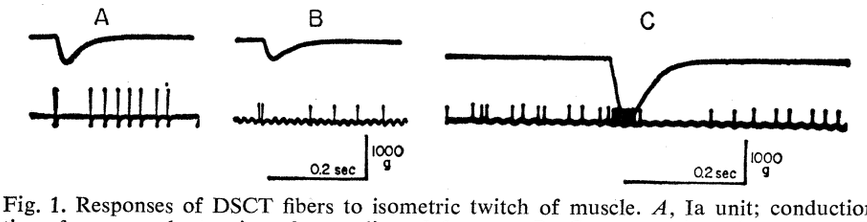

Fig. 1. Responses of DSCT fibers to isometric twitch of muscle. *A*, Ia unit; conduction time from muscle to site of recording, 3.6 msec. *B*, Gastrocnemius-soleus, group-II unit; condition time, 6 msec. *C*, Tibialis anterior–extensor digitorum longus, Ib unit; conduction time, 3 msec. Records *A* and *B* were obtained with zero initial tension; *C*, with the muscle fully extended. Spikes retouched in *A* and *B*.

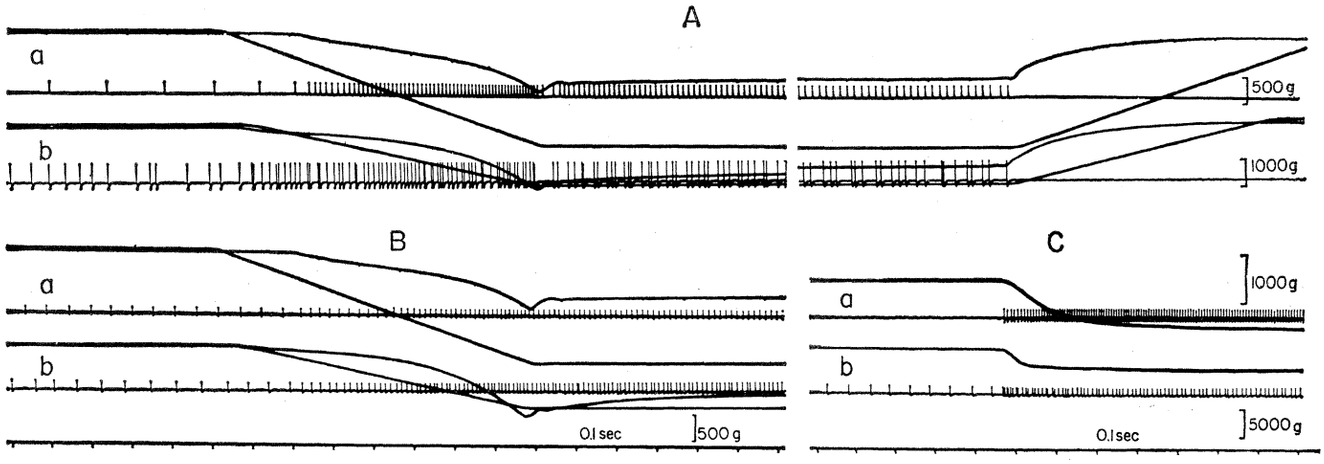

Fig. 2. Comparison of responses of primary afferent fibers (upper) and corresponding types of DSCT fibers (lower). (*A*) Ia primary afferent fiber of lateral gastrocnemius muscle (*a*) and Ia DSCT unit of gastrocnemius-soleus (*b*) during linear stretch of 18 mm/sec and release of the muscle. Periods of 1.2 seconds in *a* and 14 seconds in *b* have been cut from the middle of the static stretch. The Ia unit of *b* is the same as that of Fig. 1*A*. (*B*) Group-II primary afferent fiber of lateral gastrocnemius (*a*) and group-II DSCT unit of gastrocnemius-soleus (*b*) during 18-mm/sec linear stretch of the muscle. Conduction time of unit in *b* was 4.6 msec. Length and tension of the muscle are the additional signals in records of *A* and *B*. (*C*) (*a*) Tendon organ primary afferent fiber of soleus during tetanic contraction of the muscle elicited by stimulation of ventral root L VII; (*b*) Ib DSCT unit of gastrocnemius-soleus during tetanic contraction of the muscle elicited by stimulation of ventral root L VII. Top traces of *a* and *b* show muscle tension. Same time base in all records; spikes retouched.

in the discharge patterns of the corresponding receptors to similar stretches (Fig. 2*A*, *a*).

One striking difference between the firing patterns of the first- and second-order neurons was immediately apparent. While the firing of the primary afferent fibers was remarkably regular, with nearly constant intervals between the spikes at the initial and final lengths, the firing of the second-order neuron was characteristically irregular, often more so than the unit illustrated in Fig. 2*A*, *b*. Some Ia DSCT units, particularly those firing at a rate less than 15 impulses per second, fired "spontaneously" at a fairly regular rate. On extension of the muscle, however, the characteristic irregularity of the Ia units appeared; the degree of irregularity of firing can be assessed from the so-called coefficient of variability, which is the ratio of the standard deviation of the distribution of spike intervals to the mean interval expressed as a percentage. For one unit, the mean interval of the spontaneous discharge was about 50 msec, with a coefficient of variability of 20 percent. During full static extension the mean interval was 9.6 msec; the coefficient of variability, 35 percent. Other Ia units, however, particularly those with higher rates of "spontaneous" firing, were equally irregular during "spontaneous" activity and during stretch of the muscle. A detailed analysis of the irregularity of the spike intervals requires extensive computation and will be published later.

The response of a group-II DSCT unit is shown in Fig. 2*B*, *b*; again it is strikingly similar to the response of the corresponding sense organ (Fig. 2*B*, *a*). The frequency of firing increased progressively during the dynamic phase of the stretch, but in contrast with the behavior of the Ia units the fall in frequency at the transition to static extension was gradual and much less pronounced. This lack of "dynamic sensitivity" characterized the response of all the five group-II DSCT units studied; it provided additional evidence for the view that these units are excited by afferent fibers from the secondary endings of the muscle spindles. Another interesting feature of the response of the group-II DSCT units appears in Fig. 2*B*, *b*; intervals between impulses, during spontaneous activity as well as after stretching, were much more regular than for most group-Ia units. At full extension of the muscle the interval distribution of one of the group-II units had a coefficient of variability of only 12 percent—about one-third of the usual variability of the Ia units.

The response of the Ib DSCT units also showed striking similarity to that of the corresponding receptors, the Golgi tendon organs. Twelve of the 16 units studied were firing "spontaneously" at a rate lower than that of some of the Ia units. It may be significant that the "spontaneous" dis-

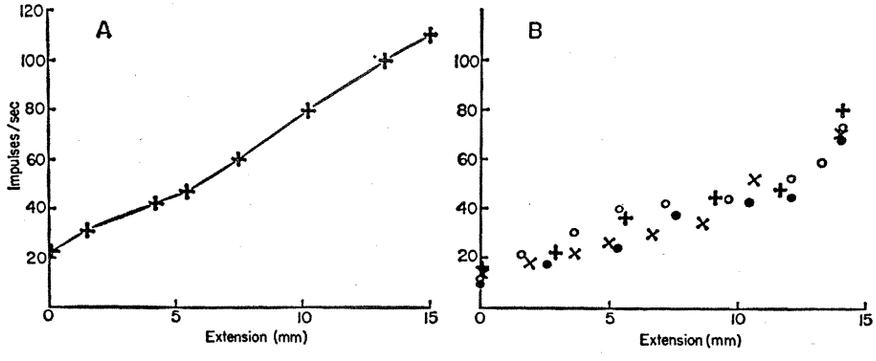

Fig. 3. Plots of mean frequency of firing (ordinate) against static extension of muscle (abscissa). (*A*) Ia DSCT unit; conduction time, 3.6 msec. (*B*) Observations from four different series of extensions on a Ia DSCT unit; conduction time, 3.8 msec. First trial, +; second trial, o; third trial, ●; fourth trial, ×.

charge was usually very regular; an example of this appears in the initial part of the record of Fig. 2*C*, *b*. The Ib units did not reach high frequencies of firing during passive extension of the muscle. This agrees with the observation that within its physiological limits the passive tension of a muscle is only a fraction of the tension that the muscle can develop during contraction, and that the tendon organs in general do not fire at high frequencies during passive extension. On the other hand, the Ib units responded vigorously to tetanic contractions of the muscle, much like the Golgi tendon organs (Fig. 2*C*, *a* and *b*). Firing rates maintained as high as 180 impulses per second were observed for Ib DSCT units during tetanic contractions; during such contractions of the muscle the Ia DSCT units characteristically reduced their firing rate as might be expected from their origins from the primary endings of the muscle spindles. Regularity of discharge of the Ib DSCT units was not usually as marked as that of the unit illustrated in Fig. 2*C*, *b*, but neither were the Ib units usually as strikingly irregular as the Ia DSCT units.

Responses of the first- and second-order neurons of this system may be usefully compared during various degrees of static extension of the muscle. Records were made during a series of stretches short of the final length, at each of which lengths the quasi-steady-state response of the ending could be determined. A plot of the response of a Ia DSCT unit against muscle length appears in Fig. 3*A*. To match the existing information on the primary afferent fibers (*6*) the response of the unit at each length was determined as the mean frequency of firing during 0.1 second, measured 0.5 second after the end of the dynamic phase of the stretch. In Fig. 3*A* the relationship between mean firing frequency and muscle length was approximately linear over the range studied, and this is a fair general representation of our observations on 20 Ia DSCT units. Although the degree of linearity varies considerably from one unit to another, there seems to be no other simple relationship that gives a better representation of our material. In particular, there was no flattening of the curve at higher frequencies as was found by Mountcastle *et al.* (*7*) in third-order thalamic neurons mediating joint receptor information. Similar observations on another Ia DSCT unit were made during four different series of interrupted stretches (shown by different symbols in Fig. 3*B*). The results demonstrate the degree of constancy of the response of DSCT units to similar stretches and confirm the approximately straight-line relationship between response and extension. The least-squares regression line of all the observations of Fig. 3*B* considered together has a coefficient of correlation of 0.88. The slope constant of the line is 3.6 impulses per second per millimeter, with 95-percent confidence limits of 3.1 and 4.1 impulses per second per millimeter.

The slope constants of the different Ia DSCT units had rather different values. The highest slope constant observed for gastrocnemius-soleus units was 8.5 impulses per second per millimeter; for TA-EDL units, 8.7 impulses per second per millimeter. Some units were only weakly excited by stretch of the muscle, having slope constants of less than one impulse per second per millimeter; such units were weakly connected to the primary endings of the muscle that was stretched, and presumably would be activated mainly by afferent fibers from muscles denervated in our experiments. Therefore, no great value can be attached to the mean value of the slope constants of the various units studied. We can state, however, that slope constants of the order of three to five impulses per second per millimeter were frequent for both gastrocnemius-soleus and TA-EDL Ia units. These values remarkably resemble the slope constants of the corresponding sense organs determined under comparable conditions (*3*, *6*).

JAN K. S. JANSEN
TORSTEIN RUDJORD
Anatomical Institute,
University of Oslo, Norway

References and Notes

1. A. Lundberg, *Progress in Brain Research* (Elsevier, Amsterdam, 1964), vol. 9, p. 135; O. Oscarsson, *Physiol. Rev.*, in press.
2. P. B. C. Matthews, *J. Physiol.* **168**, 660 (1963); J. K. S. Jansen and T. Rudjord, *Acta Physiol. Scand.* **62**, 364 (1964).
3. E. Alnæs, J. K. S. Jansen, T. Rudjord, *Acta Physiol. Scand.*, in press.
4. A. Lundberg and O. Oscarsson, *ibid.* **38**, 53 (1956); **50**, 356 (1960).
5. J. C. Eccles, O. Oscarsson, W. D. Willis, *J. Physiol.* **158**, 517 (1961).
6. J. K. S. Jansen and P. B. C. Matthews, *Acta Physiol. Scand.* **55**, 376 (1962).
7. V. B. Mountcastle, G. F. Poggio, G. Werner, *J. Neurophysiol.* **26**, 807 (1963).
8. We thank Jan Rausanaksel for technical assistance, Anders Lundberg for lending the micromanipulator, and P. M. H. Rack for comments on the manuscript.

26 April 1965

DNA: Reaction with Chloroquine

Abstract. *Difference spectrophotometry shows that double-stranded DNA produces marked changes in the absorption spectrum of chloroquine; only minor changes occur with single-stranded DNA. A DNA-chloroquine complex was demonstrated to sediment in the analytical ultracentrifuge. Chloroquine strongly elevated the thermal dissociation temperature,* T_m, *of DNA. It is concluded that the drug forms a complex with DNA by ionic interaction and stabilizes the helix.*

Certain antimicrobial substances form complexes with DNA and produce biological effects by inhibiting reactions in which DNA participates. This has been demonstrated for mitomycin C (*1*), actinomycin D (*2–4*), proflavin (*5*), daunomycin, cinerubin, chromomycin A_3, and echinomycin (*6*), as well as for miracil D (*7*), and for ethidium bromide (*8*). Chloroquine (Resochin) is also known to form complexes with DNA (*9*, *10*) and to inhibit bacterial transformation (*10*) as well as DNA synthesis, RNA syn-

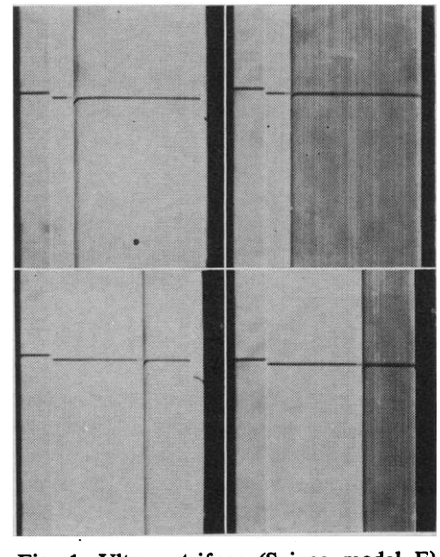

Fig. 1. Ultracentrifuge (Spinco model E) sedimentation patterns of 2 mg of calf thymus DNA (Worthington) per milliliter in the absence (left) and presence (right) of 160 μg of chloroquine per milliliter. The chloroquine used throughout these studies was a commercial preparation of the hydrochloride of the drug used for injection. The solvent was $5 \times 10^{-3}M$ tris-HCl at *p*H 7.5. The photographs were taken in light of 365-mμ wavelength, 32 minutes and 128 minutes after a speed of 59,780 rev/min had been attained. The absorbancy (A) at 365 mμ of the DNA-chloroquine mixture was 0.50 for the centrifuge cell with an optical path of 1.2 cm.