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SYNAPTIC ACTION ON CLARKE'S COLUMN NEURONES IN RELATION TO AFFERENT TERMINAL SIZE

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SUMMARY

1. Excitatory post-synaptic potentials (e.p.s.p.s) were recorded intracellularly from Clarke's column neurones (DSCT neurones) of the cat in response to adequate stimuli applied to a variety of sensory receptors.

2. The amplitude of e.p.s.p.s so produced varied from less than 0·2 mV to more than 2–3 mV. The amplitude distribution of e.p.s.p.s suggested that the mean number of 'quanta' of transmitter released by one impulse varied widely from one fibre to another arising from a given type of sensory receptor.

3. The average amplitude of e.p.s.p.s evoked by single afferent impulses was significantly smaller for cutaneous inputs than for muscle or joint inputs. However, synaptic action on DSCT neurones produced by different sensory inputs was equally greater, on the average, than that on spinal motoneurones.

4. Both small and large e.p.s.p.s in DSCT neurones failed to increase in amplitude during post-synaptic hyperpolarization applied through the cell body. This failure could not be attributed to possible anomalous rectification in the post-synaptic membrane.

5. Small and large e.p.s.p.s were comparable in half-decay time, but there was a positive correlation between amplitude and time-to-peak of e.p.s.p.s. It is suggested that the locations of synapses responsible for small and large e.p.s.p.s are intermingled on the dendrites and that large e.p.s.p.s are associated with a longer duration of transmitter action than small e.p.s.p.s.

6. Degenerating terminals of primary afferent fibres on DSCT neurones

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and motoneurones were examined with the electron microscope after chronic section of the dorsal roots.

7. Dendritic degenerating terminals showed no significant difference in size between motoneurones and DSCT neurones. Degenerating 'giant' terminals were found on DSCT neurones, but they were located only on or very close to the cell body.

8. It is concluded that the major factor responsible for a large number of 'quanta' of transmitter released at synapses on DSCT neurones is the number of multiple synaptic contacts formed by one afferent fibre rather than the size of individual synapses.

INTRODUCTION

The neurones of the dorsal spinocerebellar tract (DSCT) are located in Clarke's column and receive monosynaptic connexions from primary afferent fibres arising in a variety of sensory receptors (Lundberg, 1964; Oscarsson, 1965). Maximum electrical stimuli applied to such monosynaptic paths often produce large excitatory post-synaptic potentials (e.p.s.p.s) in DSCT neurones which range up to 30–65 mV in amplitude (Kuno & Miyahara, 1968; Eide, Fedina, Jansen, Lundberg & Vyklický, 1969*a*). This contrasts with synaptic action on spinal motoneurones in which the maximum monosynaptic e.p.s.p.s rarely exceed 12 mV (Eccles, Eccles & Lundberg, 1957). In addition, the mean amplitude of monosynaptic e.p.s.p.s evoked by activation of a single afferent fibre is also greater in DSCT neurones than in motoneurones (Eide, Fedina, Jansen, Lundberg & Vyklický, 1969*b*; also, cf. Kuno & Miyahara, 1968). The input resistance is generally higher in DSCT neurones than in motoneurones (Hongo & Okada, 1967; Kuno & Mihahara, 1968), but this difference alone is insufficient to account for the difference in the e.p.s.p. amplitude between the two groups of neurones. Thus, it has been postulated that large e.p.s.p.s evoked in DSCT neurones are at least in part due to a large number of 'quanta' of transmitter released by impulses in individual afferent fibres (Kuno & Miyahara, 1968; Eide *et al.* 1969*b*).

A substantial number of synaptic terminals on DSCT neurones are significantly larger in size ('giant' terminals) than those on motoneurones (Szentágothai & Albert, 1955; Kuno, 1969; Réthelyi, 1970). At the neuromuscular junction, the mean number of quanta of transmitter released by a nerve impulse is directly related to the size of nerve terminals (Kuno, Turkanis & Weakly, 1971). Therefore, one may be tempted to speculate that the large number of quanta released by afferent impulses at synapses on DSCT neurones may be correlated with the presence of 'giant' terminals on these neurones (Kuno & Miyahara, 1968; Eide *et al.* 1969*b*). In fact, at

least some of the 'giant' terminals were found to arise from primary afferent fibres as evidenced by degeneration following chronic section of the dorsal roots (Szentágothai & Albert, 1955). However, it remains uncertain whether primary afferent fibres form exclusively 'giant' terminals on DSCT neurones (Réthelyi, 1970). It is possible that size of nerve terminals may depend on the type of sensory fibre, so that the intensity of synaptic input to DSCT neurones may vary from one receptor type to another. Furthermore, each of the presynaptic axons has often been found to make multiple contacts on a DSCT neurone in the form of *boutons de passage* (Szentágothai & Albert, 1955; Réthelyi, 1970). A question then arises as to whether strong action on DSCT neurones may result from the large *number* of synaptic contacts arising from one afferent fibre rather than the large *size* of individual synaptic terminals (Réthelyi, 1970; Kuno, 1971).

The present study is concerned with two questions: (1) does the intensity of synaptic action on DSCT neurones depend on the type of sensory receptor activated? (2) are large e.p.s.p.s evoked in DSCT neurones by single afferent impulses correlated with the size of presynaptic terminals on these neurones?

METHODS

Physiological observations. The experiments were performed on adult cats. The results of physiological observations to be presented in this paper were obtained from the series of experiments reported in a previous study (Kuno, Muñoz-Martinez & Randić, 1973) in which technical details have already been described.

Morphological observations. Eight cats were prepared for morphological observations to measure the size of degenerating terminals of primary afferent fibres following section of the dorsal roots. The dorsal roots on the left side were sectioned intradurally from the sixth lumbar through the first sacral segments under pentobarbitone anaesthesia with aseptic precautions. After a post-operative period varying from 2 to 15 days, the cats were anaesthetized by I.P. injection of sodium pentobarbitone, and intravascular perfusion was made through the left ventricle with 4·3 % glutaraldehyde in McEwen's buffer solution (McEwen, 1956) for 50–60 min. The spinal cord was then immediately removed, and the Clarke's column region in the left third lumbar segment as well as the motoneurone region in the left seventh lumbar segment was isolated under a dissecting microscope. These tissues were further divided into small pieces of about 1 mm^3. The specimens were post-fixed in 1 % osmium tetroxide, dehydrated in alcohol and embedded in the epoxy resin (Epon). To determine the orientation of each specimen, preliminary thick sections (about 1 μm) were cut and stained with toluidine blue. The blocks were appropriately trimmed so as to contain neurone cell bodies larger than 40 μm in diameter. Ultrathin sections were stained with lead citrate and/or uranyl acetate and examined with the electron microscope. Each section was cut approximately every 5 μm in order to avoid sampling the same terminal more than once.

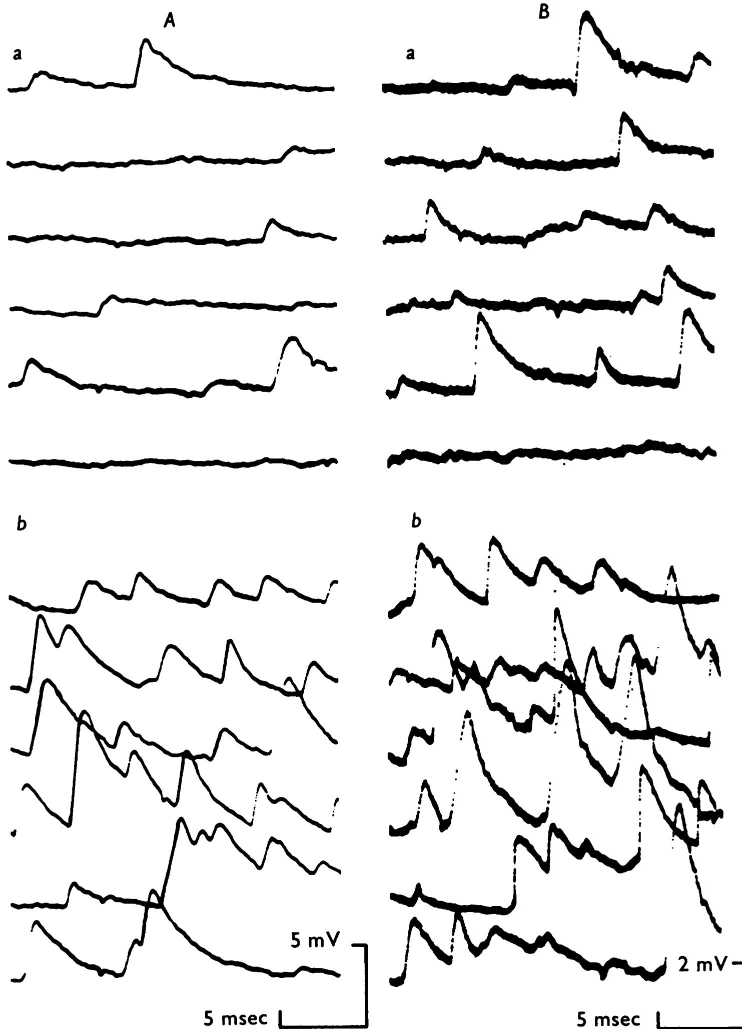
RESULTS

Physiological observations

E.p.s.p.s evoked by activation of different receptors. Text-fig. 1 illustrates e.p.s.p.s recorded intracellularly from two different DSCT neurones (*A* and *B*). One (*A*) of the neurones responded exclusively to stretch of the extensor digitorum muscle, whereas the other DSCT neurone (*B*) could be activated only by flexion and/or abduction of phalanx joints in two medial toes. Both neurones showed 'spontaneously' occurring e.p.s.p.s under 'resting' conditions (Text-fig. 1*Aa*, *Ba*). Some of the 'spontaneous' e.p.s.p.s were relatively large in amplitude (> 3 mV) and often led to firing in the neurone. Therefore, in the majority of experiments synaptic responses were routinely recorded under post-synaptic hyperpolarization (Kuno & Miyahara, 1968; Eide *et al.* 1969*b*). It was uncertain whether 'spontaneous' e.p.s.p.s were produced by background discharges of spinal interneurones or by sensory impulses initiated at the receptors in the 'resting' position. When adequate stimuli (muscle stretch or toe flexion) were applied to the sensory receptors, there was a significant increase in the frequency of e.p.s.p.s in the neurones under study (Text-fig. 1*Ab*, *Bb*). It may be assumed that the e.p.s.p.s so elicited are the responses to individual impulses asynchronously arriving over a number of sensory fibres arising from the receptors tested.

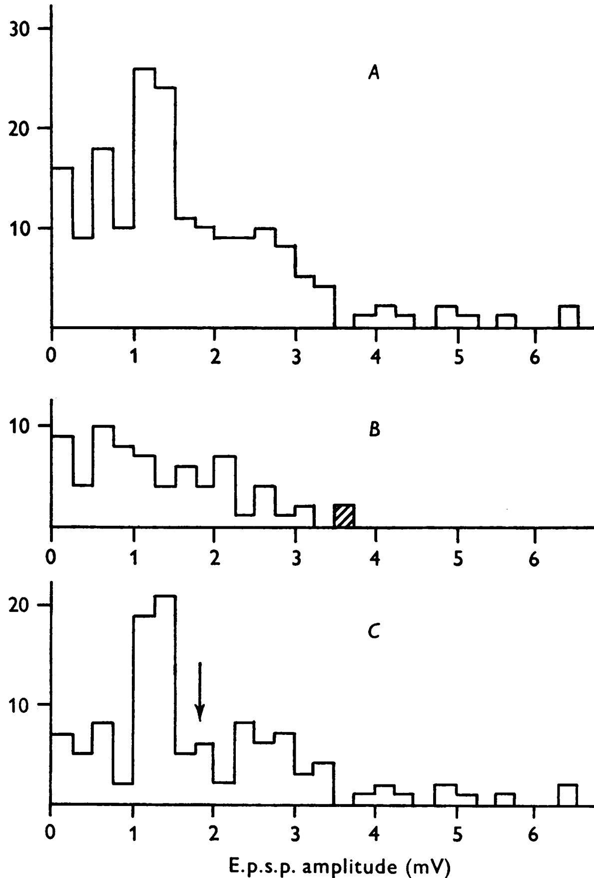
In order to discriminate individual synaptic potentials, the e.p.s.p.s occurring in DSCT neurones were recorded at relatively fast sweep speeds (Text-fig. 1). The sweeps were triggered at a rate of 1/sec, so that the e.p.s.p.s could be sampled in an essentially random manner. Text-fig. 2*A* shows the amplitude distribution of e.p.s.p.s recorded in 25 sweeps during activation of the muscle receptors. This DSCT neurone is the same one whose responses are illustrated in Text-fig. 1*A*. The e.p.s.p.s so recorded may include synaptic responses evoked by sensory impulses elicited at the receptors as well as e.p.s.p.s occurring spontaneously under 'resting' conditions. The amplitude distribution of e.p.s.p.s recorded in 25 sweeps in the 'resting' position (cf. Text-fig. 1*Aa*) is shown in Text-fig. 2*B*. The e.p.s.p. amplitude distribution constructed in Text-fig. 2*C* was obtained by subtraction of the 'resting' amplitude distribution (Text-fig. 2*B*) from the distribution of e.p.s.p.s recorded during receptor activation (Text-fig. 2*A*). The e.p.s.p.s remaining after subtraction may be assumed to reflect only synaptic responses evoked by afferent impulses initiated at the receptors. These 'evoked' e.p.s.p.s showed a wide variation in amplitude, ranging from less than 0·2 mV to more than 6 mV (Text-fig. 2*C*). The mean e.p.s.p. amplitude (arrow in Text-fig. 2*C*) was about 1·8 mV in this particular experiment.

It could be argued that large e.p.s.p. (> 1 mV) may be the results of random coincidence of several small e.p.s.p.s (< 0·2–0·4 mV). However, with the sweep speeds used in the present study, potentials occurring 1 msec apart were easily resolvable into two separate events (cf. Text-fig. 1). Therefore, even at the maximum frequency of e.p.s.p.s observed



Text-fig. 1. E.p.s.p.s recorded from two different DSCT neurones (*A*, *B*). *a*, e.p.s.p.s spontaneously occurring in the absence of added adequate stimulation. *Ab*, e.p.s.p.s recorded during activation of sensory receptors by stretch of the extensor digitorum muscle. *Bb*, e.p.s.p.s recorded during receptor activation by flexion of phalanx joints of the toes. Both DSCT neurones were hyperpolarized by the application of currents through the intracellular electrode in order to prevent initiation of action potentials.

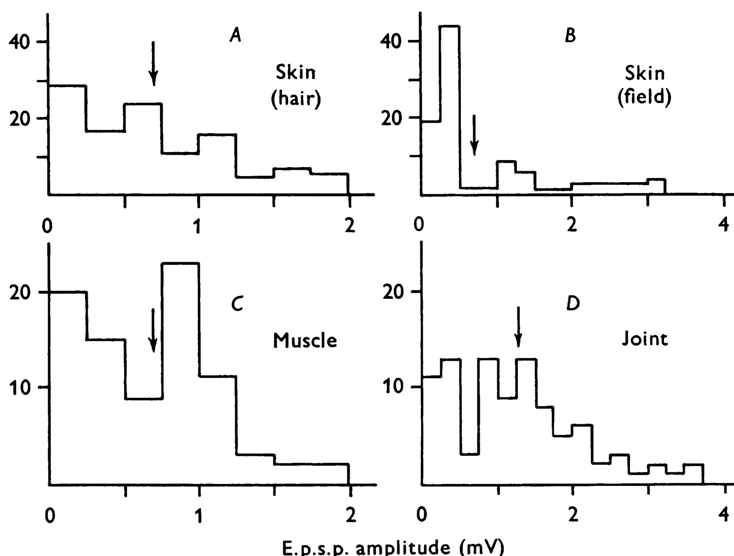
(300/sec), the chance of more than five small e.p.s.p.s coinciding (to make up an e.p.s.p. larger than 1–2 mV) would be less than 0·6 %. Also, large e.p.s.p.s ranging up to about 3 mV were observed under 'resting' conditions (Text-fig. 2*B*) in which the frequency of e.p.s.p.s was too low to give



Text-fig. 2. Amplitude distributions of e.p.s.p.s recorded from the cell illustrated in Text-fig. 1*A*. *A*, for e.p.s.p.s recorded during receptor activation. *B*, for e.p.s.p.s recorded in the absence of added adequate stimulation. *C*, amplitude distribution constructed by subtraction of *B* from *A*. Arrow, mean amplitude.

rise to coincident occurrence (cf. Text-fig. 1*Aa*, 1*Ba*). Therefore, large e.p.s.p.s cannot be attributed to summation of e.p.s.p.s evoked by synchronously arrived sensory impulses. It is concluded that the amplitude of e.p.s.p.s produced in DSCT neurones by single sensory impulses varies over a tenfold range. In a few experiments, the number of e.p.s.p.s of a

particular amplitude observed under 'resting' conditions (shaded column in Text-fig. 2*B*) was slightly larger than that seen during receptor activation. These e.p.s.p.s were ignored, but the number of e.p.s.p.s thus discarded was so small that the error induced by this procedure did not exceed 1 % for the estimate of the mean e.p.s.p. amplitude.



Text-fig. 3. Same as Text-fig. 2*C*, but for four different DSCT neurones activated by hair movement (*A*), activation of field receptors on the skin (*B*), pressure on the gluteal muscle (*C*) and activation of joint capsule in the knee (*D*). Arrows, mean amplitudes.

Text-fig. 3 illustrates the results obtained by similar subtraction methods from four different DSCT neurones activated by various sensory receptors. Adequate stimuli to the skin were applied manually by rubbing the receptive field after the receptor type had been determined. Muscle receptors were activated either by stretch of the muscle or by pressure applied to the muscle belly. Activation of joint receptors was produced by pressing the exposed joint capsule with a glass rod or by passive movements of the joint. Under these conditions, the frequency of e.p.s.p.s evoked by receptor activation may depend on the number of afferent fibres activated as well as on the discharge frequency of individual fibres. If the mean number of quanta of transmitter released by one impulse is approximately the same for different fibres arising from a given type of sensory receptor, the e.p.s.p. amplitude distribution should be described by a certain statistical law (normal, binomial or Poisson), depending on the

statistical nature of transmitter release (cf. Blankenship & Kuno, 1968). However, the amplitude distributions fit none of these statistical distributions (Text-fig. 3), and there was no consistent amplitude distribution for different experiments. Thus, the present results suggest that the amplitude of e.p.s.p.s in a DSCT neurone in response to single afferent impulses varies markedly from fibre to fibre arising from the same type of sensory receptor. Therefore, the e.p.s.p. amplitude distribution is different in different experiments, depending on the properties of sensory fibres involved during receptor activation. This notion agrees with previous observations that stimulation of a single afferent fibre produced large e.p.s.p.s (about 3·5 mV) in a DSCT neurone, whereas stimulation of another fibre evoked small e.p.s.p.s (0·4–1·4 mV) in the same neurone (Kuno & Miyahara, 1968). It has also been noted that the mean amplitude of e.p.s.p.s generated by activation of single afferent fibres arising from the muscle varies over a tenfold range from fibre to fibre (Eide *et al.* 1969*b*).

Arrows in Text-fig. 3 represent the mean amplitudes of e.p.s.p.s (also, cf. Text-fig. 2*C*). It is evident that the mean e.p.s.p. amplitude is determined by the relative proportions of small and large e.p.s.p.s in each test. Therefore, if there is a significant difference in the relative proportions of sensory fibres which may produce varying degrees of synaptic action on DSCT neurones, the mean e.p.s.p. amplitude would be different for activation of various sensory receptors. The observations were made on eleven DSCT neurones activated by muscle receptors (muscle group), seven DSCT neurones activated by skin including subcutaneous receptors (cutaneous group) and five DSCT neurones activated by joint receptors (joint group). The averages of the mean e.p.s.p. amplitudes were 1·1 mV (with a range of 0·4–1·8 mV) for muscle group, 0·7 mV (with a range of 0·5–1·1 mV) for cutaneous group and 1·4 mV (with a range of 0·8–2·3 mV) for joint group. The mean e.p.s.p. amplitude of cutaneous group was significantly ($P < 0{\cdot}05$) smaller than that of muscle or joint group, whereas the difference between muscle and joint groups was statistically insignificant. However, it should be noted that the mean amplitude of e.p.s.p.s evoked in motoneurones by single afferent impulses are 0·1–0·3 mV (Kuno & Miyahara, 1969; Mendell & Henneman, 1971; Jack, Miller, Porter & Redman, 1971). It may be concluded that synaptic action on DSCT neurones produced by different inputs is equally greater, on the average, than that on spinal motoneurones.

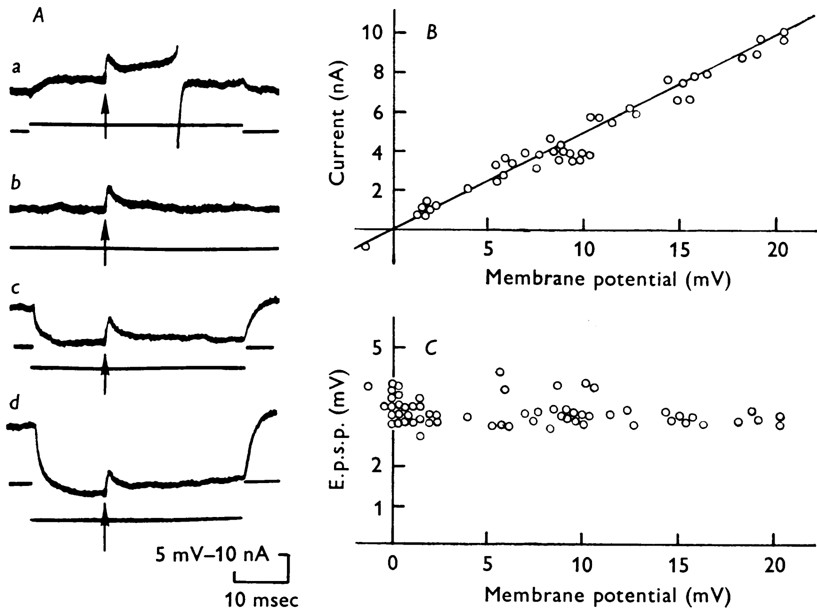
Effects of post-synaptic polarization. One of the principal questions posed in the present study was whether a group of large e.p.s.p.s (> 1–2 mV) observed in every DSCT neurones tested is correlated with the presence of 'giant' terminals on these neurones. It has been shown that 'giant'

terminals on DSCT neurones are located on or close to the cell body (Szentágothai & Albert, 1955; Réthelyi, 1970; also, see below). If the large e.p.s.p.s are generated at 'giant' synapses, one may expect that the amplitude of large e.p.s.p.s would be sensitive to the changes in the membrane potential produced by post-synaptic polarizing currents applied through the cell body (see, Rall, 1960, 1964, 1967). The amplitude distributions of 'evoked' e.p.s.p.s (cf. Text-fig. 2*C*) were obtained before and during the application of post-synaptic hyperpolarizing currents (20–45 nA) in eight DSCT neurones. All neurones showed no significant changes in the mean e.p.s.p. amplitude as well as in the range of the distribution by the application of post-synaptic hyperpolarization.

It may be argued that the input resistance of DSCT neurones may decrease during hyperpolarization (Gustafsson, Lindström & Takata, 1971; but cf. Kuno & Miyahara, 1968; Kuno, Miyahara & Weakley, 1970) and that such anomalous rectification may mask the effect of post-synaptic hyperpolarization on the e.p.s.p. amplitude (Kandel & Tauc, 1966; Nelson & Frank, 1967; Eide *et al.* 1969*a*). This possibility was tested in the experiment illustrated in Text-fig. 4. Monosynaptic e.p.s.p.s were evoked in a DSCT neurone by stimulation of a single afferent fibre dissected from the dorsal root (cf. Kuno & Miyahara, 1968). The e.p.s.p.s so produced showed a random fluctuation in amplitude, ranging from about 3 to 4 mV, at the resting membrane potential (at 0 in Text-fig. 4*C*). Text-fig. 4*A* shows the effect of depolarizing (*a*) and hyperpolarizing (*c*, *d*) current pulses applied through the cell membrane. The changes in the membrane potential were apparently proportional to the applied current intensities, and there was no indication of anomalous rectification (Text-fig. 4*B*). However, the e.p.s.p.s again showed no detectable increase in amplitude during post-synaptic hyperpolarization (Text-fig. 4*C*). It is suggested that the synapses responsible for the generation of large e.p.s.p.s may be located remotely on the dendrites, so that polarizing currents applied through the cell body may be subjected to electronic attenuation before reaching the synapses under study (Rall, 1960, 1964, 1967).

Time course of e.p.s.p.s. The location of synaptic input proximal or distal to the cell body may also be distinguished by the time-to-peak and/or the decay time of e.p.s.p.s (Rall, 1960, 1967). The inset of Text-fig. 5 illustrates e.p.s.p.s recorded from a DSCT neurone during activation of joint receptors. The frequency of e.p.s.p.s was appropriately controlled by adjusting the intensity of adequate stimuli, so that each e.p.s.p. could be clearly separated without interference due to summation. It was thus possible to measure the time-to-peak and half-decay time of individual e.p.s.p.s of various amplitudes. Text-fig. 5*B* shows the relation between the amplitude and the half decay time for over ninety e.p.s.p.s recorded

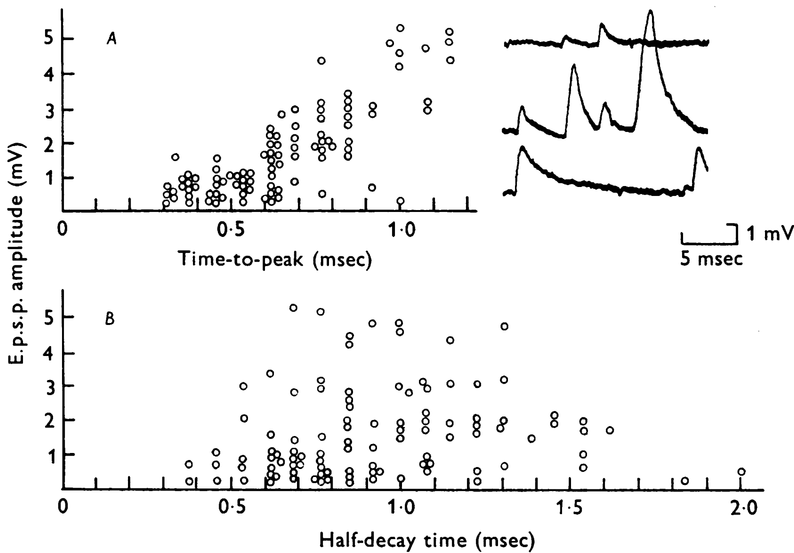
from the same DSCT neurone. Although the points showed a considerable scatter, there was no clear difference in half-decay time between small and large e.p.s.p.s (Text-fig. 5*B*). Therefore, it is unlikely that the locations of synapses responsible for large e.p.s.p.s are distinct from those responsible for small e.p.s.p.s. In contrast, there was a positive ($P < 0{\cdot}01$) correlation



Text-fig. 4. *A*, monosynaptic e.p.s.p.s (arrows) evoked in a DSCT neurone by stimulation of a single afferent fibre dissected from the dorsal root. *a*, during post-synaptic depolarization. *b*, at the resting membrane potential. *c*, *d*, during post-synaptic hyperpolarization. *B*, relation between the amount of current applied and the change in the membrane potential. Positive, hyperpolarization. *C*, relation between e.p.s.p. amplitude and the change of the membrane potential. Positive in membrane potential, hyperpolarization.

between amplitude and time-to-peak as shown in Text-fig. 5*A*. It is possible that large e.p.s.p.s may be associated with a longer duration of transmitter action (longer time course of synaptic current) than small e.p.s.p.s. It has been computed that, when the duration of transmitter action is prolonged from 0·1 to 0·2 m sec, the time-to-peak of e.p.s.p.s may increase by 75 %, whereas the half-decay time may increase only by 6 % (Rall, Burke, Smith, Nelson & Frank, 1967). Thus, the present results suggest that the sites of synapses responsible for small and large e.p.s.p.s in DSCT neurones are intermingled along the dendrites and that large

e.p.s.p.s are probably associated with a longer duration of transmitter action than small e.p.s.p.s.



Text-fig. 5. DSCT neurone activated from joint inputs. Inset, sample records of e.p.s.p.s taken during activation of joint receptors. *A*, relation between amplitude and time-to-peak of e.p.s.p.s. *B*, relation between amplitude and half-decay time of e.p.s.p.s.

Morphological observations

General features of degenerating terminals. The questions tested by morphological observations were twofold, (1) whether terminals of primary afferent fibres on DSCT neurones are significantly larger in size than those on motoneurones and (2) whether 'giant' terminals have a certain preferential synaptic location on DSCT neurones. For these purposes, degenerating terminals on DSCT neurones and motoneurones were examined with the electron microscope following chronic section of the dorsal roots (see Methods). One of the major criteria used for identification of degenerating terminals was the presence of electron-dense material (Pl. 1) which apparently extended to or from the presynaptic axon (Pl. 1*C*). Mitochondria in degenerating terminals generally increased in number compared with those in normal terminals (Pl. 1). These alterations are essentially similar to those reported for degenerating terminals in the central nervous system (Colonnier & Gray, 1962; Walberg, 1963; Smith & Rasmussen, 1965; McMahan, 1967; but cf. Conradi, 1969). Degenerating terminals identified by the above criteria were observed in three cats two or three days after dorsal rhizotomy. At the third day stage, many

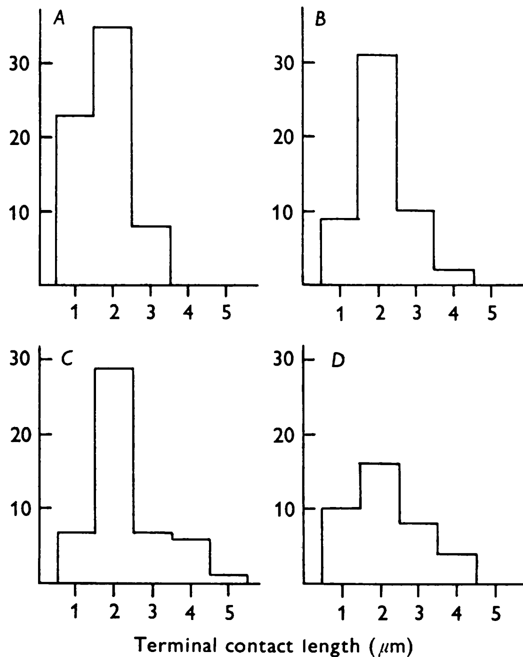
degenerating terminals were found to be engulfed in glia cells (Colonnier & Gray, 1962). These terminals were excluded from the present analysis because the length of their synaptic contacts on the post-synaptic membrane (see below) could not be measured. In the animals prepared in post-operative periods from 4 to 15 days (five cats), no degenerating terminals could be detected with certainty.

The number of degenerating terminals was extremely limited even on second and third days after section of the dorsal roots. In total, 117 degenerating terminals were found in 267 sections taken from the Clarke's column region, and 131 degenerating terminals were obtained from 111 sections taken from the motoneurone region. Often, two or three degenerating terminals were observed on the same dendrite in both DSCT neurones and motoneurones. The location of degenerating terminals was classified into somatic (Pl. 1*A*, *C*) and dendritic (Pl. 1*B*, *D*) regions, based on the presence or absence of the granular endoplasmic reticulum (er in Pl. 1*A*, *C*) in the post-synaptic neurone at the synaptic contacts. This criterion was useful to identify the cell body, particularly when the nucleus was not seen in the section. However, the granular endoplasmic reticulum may exist in large dendrites proximal to the neurone cell body (Peters, Palay & Webster, 1970), so that somatic synaptic inputs defined in the present study might have included some dendritic terminals located close to the soma. In DSCT neurones, about 23% of observed degenerating terminals were somatic, whereas somatic degenerating terminals were 11% in motoneurones.

Size distribution of terminals. The size of degenerating terminals was measured in terms of the length of synaptic contacts on the post-synaptic membrane (arrows and dotted lines in Pl. 1). The orientation of section was difficult to determine for somatic synapses. However, the direction of section of dendritic synaptic terminals could be easily determined by the arrangement of microtubules which run parallel to the length of the dendrite. Thus, longitudinally (Pl. 1*B*) and transversely (Pl. 1*D*) sectioned dendritic terminals were lumped separately. Text-fig. 6*A* shows the size distribution of transversely sectioned dendritic degenerating terminals on motoneurones, whereas the size of longitudinally sectioned terminals on motoneurones is given in Text-fig. 6*C*. Similar size distributions were constructed for transversely (Text-fig. 6*B*) and longitudinally (Text-fig. 6*D*) sectioned degenerating dendritic terminals on DSCT neurones. It is clear that transversely and longitudinally sectioned terminals are similar in contact length and that dendritic terminals show no significant difference in size between motoneurones and DSCT neurones.

It is possible that size of dendritic terminals may be related to the synaptic site along the dendrite. To test this possibility, attempts were

made to measure the diameter of the dendrite on which degenerating terminals made synaptic contact. Because of the taper of dendrites, the diameter of the dendrites may be considered to be inversely related to the distance from the cell body. Text-fig. 7*A* shows the relation between the contact length of transversely sectioned terminals on motoneurones (open circles) and DSCT neurones (filled circles) and the diameter of dendrites at

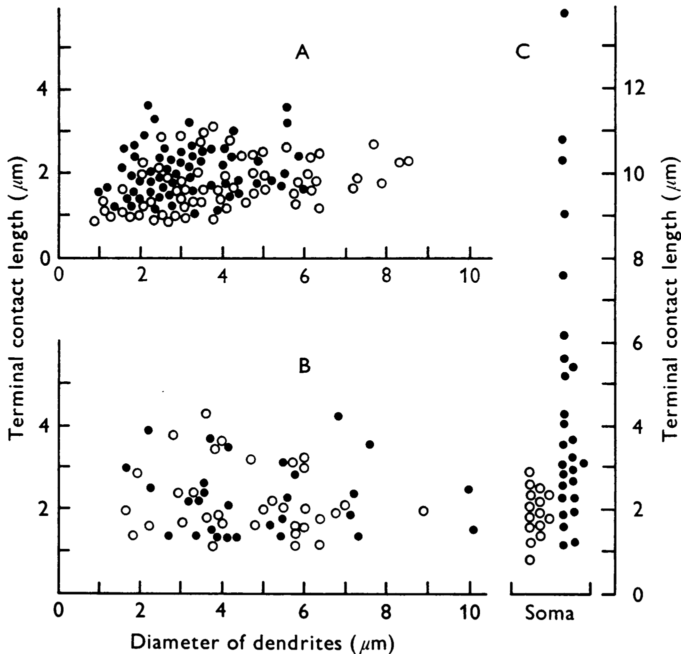


Text-fig. 6. Distribution of the contact length of degenerating dendritic terminals on the post-synaptic membrane. *A*, for transversely sectioned terminals on motoneurones. *B*, for transversely sectioned terminals on DSCT neurones. *C*, for longitudinally sectioned terminals on motoneurones. *D*, for longitudinally sectioned terminals on DSCT neurones.

the synaptic contact. The relation is similarly tested for longitudinally sectioned degenerating terminals on motoneurones (open circles) and DSCT neurones (filled circles) in Text-fig. 7*B*. Evidently, there was no indication that presynaptic terminals with a particular size have a certain preferential location along the dendrite.

Text-fig. 7*C* shows the contact length of presynaptic terminals located on the cell bodies of motoneurones (open circles) and DSCT neurones (filled circles). In motoneurones, somatic and dendritic terminals were comparable in size (Text-fig. 7, open circles), ranging from about 1 to

3–4 μm. In contrast, somatic terminals on DSCT neurones ranged from about 1 to 14 μm in contact length (Text-fig. 7*C*, filled circles). Thus, there was no doubt that DSCT neurones receive 'giant' terminals from certain primary afferent fibres (Szentágothai & Albert, 1955; Réthelyi, 1970), but these large terminals were apparently localized on or very close to the



Text-fig. 7. Degenerating terminals on motoneurones (open circles) and DSCT neurones (filled circles), *A*, *B*. Relations between contact length of dendritic terminals and the diameter of dendrites at synaptic site. *A*, for transversely sectioned terminals. *B*, for longitudinally sectioned terminals. *C*, contact length of degenerating terminals on the cell bodies.

cell body. The relative proportion of 'giant' terminals ($> 5\ \mu$m) was approximately 8 % of the total number of degenerating terminals observed on DSCT neurones. However, it should be noted that this estimate provides only the lower limit of the relative proportion of 'giant' terminals because it remains uncertain whether some of the observed small degenerating terminals may reflect a portion of sectioned 'giant' terminals.

DISCUSSION

From the present morphological observations, it seems clear that 'giant' presynaptic terminals arising from primary afferent fibres are relatively limited in number and are located only on or close to the cell body of DSCT neurones. It is possible that the majority of 'giant' terminals may arise from large afferent fibres (Szentágothai & Albert, 1955) and that large fibres may take a longer period to complete axon degeneration than small fibres (van Crevel & Verhaart, 1963). However, in post-operative periods from 4 to 15 days, the search failed to detect any degenerating terminals. One may also argue that 'giant' terminals on the dendrites may be difficult to detect without the use of serial section. This argument seemed unjustified because *normal* 'giant' terminals could be observed on the dendrites as well as on the cell body of DSCT neurones after chronic section of the dorsal roots. It is likely that these *normal* 'giant' terminals might have arisen from afferent fibres in the dorsal roots which were left intact in the present study. In fact, Réthelyi (1970) has reported that no 'giant' terminals were detected in the Clarke's column region at the third lumbar segment when unilateral dorsal rhizotomy was made from the fourth lumbar to all sacral segments. The extensive dorsal rhizotomy was not employed in the present study because our primary interest was to correlate morphological observations with the results of physiological experiments in which the major sensory inputs tested were limited from the sixth lumbar to the first sacral segments.

The physiological studies have shown that large e.p.s.p.s as well as small e.p.s.p.s evoked in DSCT neurones by single afferent impulses are not influenced by post-synaptic hyperpolarization applied through the cell body. Also, the half-decay time of large e.p.s.p.s was similar to that of small e.p.s.p.s. Thus, it appears that the sites of synapses responsible for small and large e.p.s.p.s are both located on the dendrites some distance from the cell body. This implies that the generation of large e.p.s.p.s in response to single afferent impulses is not directly related to the presence of 'giant' terminals observed on the cell body.

There was no significant difference in the size of dendritic terminals between motoneurones and DSCT neurones. However, e.p.s.p.s evoked in DSCT neurones by single sensory impulses were appreciably greater in amplitude than those produced in motoneurones, regardless of the type of receptor activated. Réthelyi (1970) has suggested that large e.p.s.p.s observed in DSCT neurones may be related to multiple synaptic contacts formed by a single sensory fibre rather than to the size of individual synapses (also, cf. Kuno, 1971). This suggestion is compatible with the present results. It is likely that the duration of transmitter action may be

prolonged by conduction time of an afferent impulse along the terminal axon which makes multiple (parallel) contacts on a DSCT neurone in the form of *boutons de passage*. This conduction time is probably the order of a fraction of a msec, but such a small increase in the duration of transmitter action is sufficient to account for a significant increase in time-to-peak without detectable changes in half-decay time of e.p.s.p.s (Rall *et al.* 1967). It may be assumed that each of the dendritic contacts is similar in size (Text-fig. 6) and that an afferent fibre with a longer terminal axon has a greater number of multiple contacts. Under these conditions, both amplitude and time-to-peak of individual e.p.s.p.s would be proportional to the number of contacts arising from a single afferent fibre. It is suggested that the number of synaptic contacts formed by one afferent fibre is the major determinant for the mean number of quanta of transmitter released at synapses on DSCT neurones. This suggestion, of course, does not conflict with the notion that the mean number of quanta of transmitter is directly related to the size of individual nerve terminals (Kuno *et al.* 1971). A number of small terminals arising from one fibre would be essentially the same in effect as a single 'giant' terminal (Kuno, 1971). 'Giant' terminals observed on the cell body may also be capable of releasing a large number of quanta. However, the present study failed to detect those e.p.s.p.s which may be generated by synapses on the cell body. This might have been simply due to the relatively small proportion of somatic synapses formed by afferent fibres.

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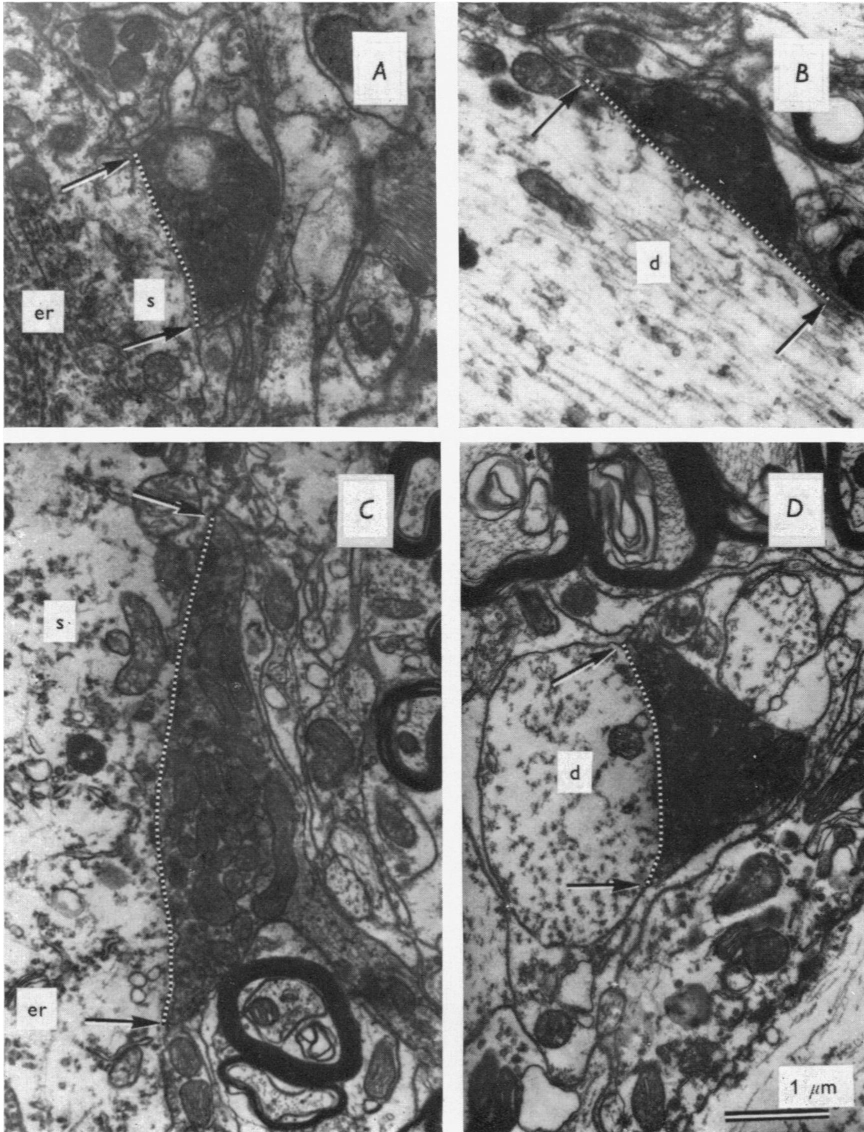
EXPLANATION OF PLATE

Electron microscopic pictures of degenerating presynaptic terminals. Synaptic contacts of degenerating terminals on the post-synaptic membrane are shown by dotted lines and arrows. *A*, motoneurone cell body. s, soma. er, granular endoplasmic reticulum. Two days after dorsal root section. *B*, motoneurone dendrite. d, dendrite. Two days after dorsal root section. *C*, Clarke's column neurone cell body. s, soma. er, granular endoplasmic reticulum. Two days after dorsal root section. *D*, Clarke's column neurone dendrite. d, dendrite. Two days after dorsal-root section.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