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# THE EXCITATION OF RENSHAW CELLS BY PHARMACOLOGICAL AGENTS APPLIED ELECTROPHORETICALLY

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It has been reported that a group of cells lying within the ventral horn of the lumbar segments of the spinal cord of the cat are normally discharged by the release of acetylcholine from the terminals of the motor axon collaterals (Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956). These cells, the Renshaw cells, in turn inhibit adjacent motoneurones. Some of the pharmacological properties of these pathways have already been investigated (Curtis, Eccles & Eccles, 1957). The anomalous behaviour of these cells when tested with certain drugs, specifically acting on peripheral cholinergic synapses, given either intravenously or intra-arterially, has suggested that they are surrounded by at least two diffusional barriers. In order to investigate these barriers more closely, methods have been devised by which drugs can be applied directly from a micro-electrode in the vicinity of the cell. These methods have been used in an investigation of the neuromuscular junction (del Castillo & Katz, 1955; 1957*a–c*).

## METHODS

All experiments have been performed upon Renshaw cells located in the lower lumbar segments of the spinal cord of the cat under light pentobarbital anaesthesia. Since these cells are often difficult to locate, it was important to be able to test any one cell with a variety of pharmacological agents, and therefore five-barrelled electrodes were used. Four barrels, each containing a different drug in solution, specified in Table 1, were arranged radially around a central barrel containing 4M-NaCl, which was employed as the electrode recording the responses of the cell. Renshaw cells were located by finding areas within which the responses of a single cell to impulses in motor axon collaterals could be recorded. These impulses were set up by stimulating the cut ventral root of the appropriate segment (i.e. orthodromic or synaptic stimulation of the Renshaw cell). The composite electrode was held in a micromanipulator (Eccles, Fatt, Landgren & Winsbury, 1954), and once located the responses of a single cell could be recorded extracellularly for 3–4 hr. The spikes recorded were within the range 1·5–0·4 mV.

It was possible to apply positive or negative voltages either constantly or in pulses to any of the four drug-containing barrels. The voltage on each barrel was monitored by means of a cathode follower and the current flowing through the barrels could be recorded either by a galvanometer or on one beam of the oscilloscope after amplification. The current-carrying lines were connected via resistances of 100 MΩ (cf. Coombs, Eccles & Fatt, 1955) and chlorided silver wires to the barrels

containing the drugs. A small negative backing voltage of 200–300 mV was applied to each barrel to prevent diffusion of the active cation from the tips (del Castillo & Katz, 1955).

Care had to be taken in these experiments that the electrical resistance of any one barrel was not too high. The recording barrel was connected to another cathode follower by a chlorided silver wire and the total input capacity of the system was at least 30 pF. Consequently, the electrode resistance had to be kept low (between 4 and 10 MΩ) in order to record the rapid spike potentials of Renshaw cells without considerable distortion. However, it was more important to have drug-containing barrels of low resistance. Not only does the resistance of micro-electrodes often increase considerably while passing through the spinal cord, but also resistance changes occur during the passage of current through them (Coombs, Curtis & Eccles, unpublished observations). Both these effects are more marked with electrodes of high resistance and consequently the resistance of the drug-containing barrels usually was kept below 100 MΩ. It was of special importance that the resistance of the barrel containing acetylcholine be kept constant because, as will be seen in the next paper (Curtis & Eccles, 1958), the usual testing procedure for blocking and potentiating drugs was to apply a constant voltage to the acetylcholine electrode for 3–4 sec at different testing intervals. Only if the resistance was constant could the same dose of acetylcholine be delivered on these different occasions.

TABLE 1. Drugs used, approximate concentrations and molecular weights

| | Concn. (M) | mol. wt. (approx.) |
|---|---|---|
| Acetylcholine bromide | 1–2 | 226 |
| Nicotine hydrochloride | 7–10 | 235 |
| Carbaminocholine chloride | 0·5 | 182·7 |
| Acetyl-β-methylcholine | 3 | 195·7 |
| Arecholine hydrobromide | 0·25 | 236 |
| Succinylcholine bromide | 0·25 | 155 |
| Tubocurarine chloride | 0·04 | 785·7 |
| Procaine hydrochloride | 1 | 272·8 |
| Dihydro-β-erythroidine bromide | 0·5 | 250 |
| Decamethonium iodide | 0·14 | 512 |
| Prostigmine bromide | 0·2 | 303·2 |
| Edrophonium chloride (Tensilon, Roche Products Ltd.) | 0·16 | 246 |

The electrodes were first filled by boiling in distilled water, which was then extracted by suction with a fine Polythene tube and replaced from above with the drug solution. At least 48 hr were allowed to elapse before use. This method was preferred to that of del Castillo & Katz (1957*a*), which depended on the prior use of 3 M-KCl solution, because measurements of the resistance of each barrel gave an indication as to when the drug was uniformly distributed in it. The tips of the multibarrel electrodes were broken off at the required diameter (1–2 μ) with a fine glass rod during microscopic observation. Most drugs were used in nearly saturated solution. The approximate concentrations and molecular weights are shown in Table 1. Electrodes containing succinylcholine were stored at 5° C, thus reducing the rate of break-down of this substance (Fraser, 1954).

Although it is possible to give the total quantity of electricity used in passing quantities of drugs from the tips of electrodes (cf. del Castillo & Katz, 1957*a*), these values are only of use when comparing the responses of a single unit to drugs passed from one electrode. The distance from the electrode tip to any one cell was indeterminate and varied from cell to cell. It was certainly much greater than the distance between the orifices of individual barrels. Since the coupling resistances between any two barrels (cf. Coombs *et al.* 1955) were of the order of 5–10 KΩ and as the currents passed rarely exceeded $50 \times 10^{-9}$ A, the interaction between barrels was negligible.

## RESULTS

### *Random activity and synaptic stimulation*

When the appropriate ventral root was stimulated antidromically, the normal type of response was obtained from Renshaw cells. The initial frequency of 700–1000/sec decreased over the first 10–50 msec (Fig. 1*A*, *C*), the actual duration of the discharge varying considerably from cell to cell. By using repetitive stimuli 10–20 msec apart it was possible to drive a single Renshaw cell to discharge at frequencies of 800–1000/sec for many seconds. This observation, together with the fall in frequency observed with a single stimulus,

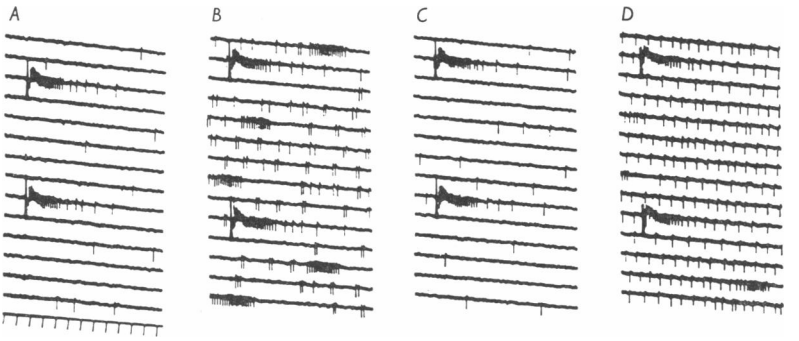


Fig. 1. The responses of Figs. 1, 2, 3*D–F* and 5 were recorded on moving film at such a rate that each sweep occupied approximately 130 msec and the delay between sweeps was less than 5 msec. *A*, *C*, random responses together with discharges evoked by maximal synaptic stimuli. The individual high-frequency responses are not clearly apparent owing to the slow sweep speed used. *B*, as for *A* and *C*, but simultaneously a current of $5 \times 10^{-8}$ A was passing through a barrel containing acetylcholine. *D*, as for *B* but a current of $10 \times 10^{-8}$ A flowed through a barrel containing nicotine. In both *B* and *D* the drug-evoked frequency was maximal. Time marker, 10 msec.

suggests that the observed response to synaptic stimulation is due to the fall in concentration of a synaptic transmitter which is initially released in a large amount, rather than to the cell becoming insensitive to the transmitter.

Most Renshaw cells discharge spontaneously and several types of random response occur. Single spikes are illustrated in Fig. 1*A*, *C*. Some cells discharged in units of two or three (Fig. 2*A*) and occasionally bursts of impulses occurred having a frequency and duration not unlike that obtained by synaptic stimulation (Fig. 1*B*). This phenomenon was more apparent during the application of acetylcholine (Fig. 1*B*) and nicotine (Fig. 1*D*). All types of spontaneous or background discharge were suppressed for some time after a response to a synaptic stimulus. In Fig. 2*A*, *D* the spontaneous spikes are absent for 0·3–0·5 sec after such a response and also in Fig. 1*A*, *C*, where the background discharge is lower. When the discharge rate was increased, either

as in Fig. 1*B* by acetylcholine or as in Fig. 1*D* by nicotine, this depression was also apparent but was of shorter duration, especially when the background rate was high (Fig. 1*D*).

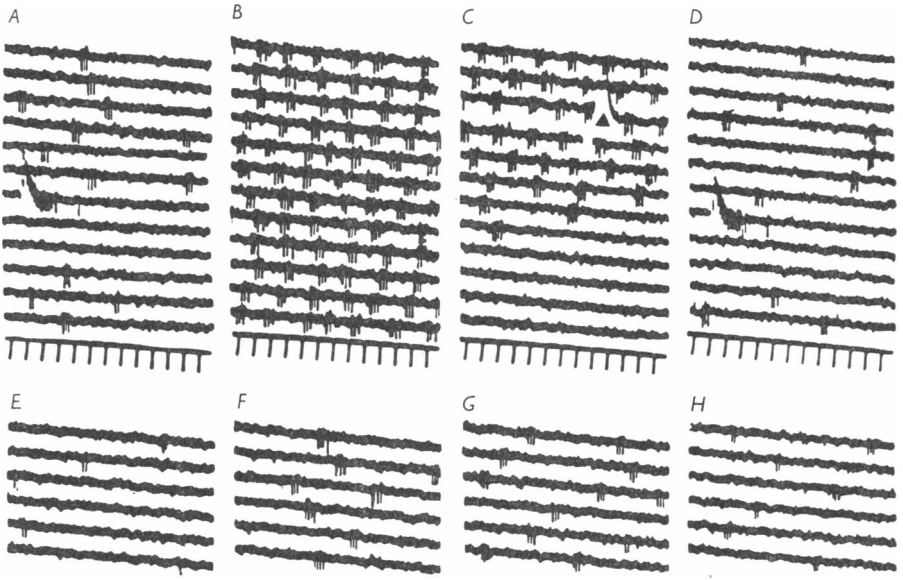


Fig. 2. All responses are from the one cell. *A*, background discharges before and after one synaptic stimulus. *B*, *C*, *D*, continuous records; in *B* and *C* (up to ▲) $3 \times 10^{-8}$ A passed through the acetylcholine barrel; one synaptic stimulus in *D*. *E*, background discharge. *F*, recorded whilst $3 \times 10^{-8}$ A flowed through a barrel containing acetyl-β-methylcholine. *G*, 4 sec after this current ceased. *H*, 30 sec after *G*.

### *Acetylcholine*

In contrast to the variable responses found when acetylcholine is administered intra-arterially (Eccles *et al.* 1956; Curtis *et al.* 1957), all Renshaw cells have responded to acetylcholine which was released locally, either by removing the backing voltage or by applying a positive voltage to the electrode. When a cell was found, the backing voltage was usually reduced until it just prevented emission of acetylcholine by diffusion, as gauged by the effect on the cell. This was important, especially when repetitive short pulses of acetylcholine were used, because the magnitude of the backing voltage has a considerable effect upon the rate at which acetylcholine leaves the tip of the electrode. No accurate estimate could be made of the distance between the tip of the electrode and the cell because, in order to avoid damaging the cell, once reasonable discharges were obtained in response to synaptic stimulation and to acetylcholine the electrode was not moved.

The duration of the application of acetylcholine could be measured accurately, but because of the latency of the response, brief pulses (less than

0·1 sec) were rarely used. Usually the current was applied by a manually operated switch for 3–9 sec. In those cells responding with short latency, brief pulses of acetylcholine were released, and in several instances the minimal doses for single responses of individual cells was approximately $10^{-14}$ equiv of acetylcholine (del Castillo & Katz, 1955). The minimum latency varied considerably over a range 30–1000 msec and was least for any one cell when large doses were used. Most cells responded with repetitive discharges of single impulses (Fig. 3*A*, *B*) but with some the impulses were grouped in doubles or even in triples (Figs. 1*B*, 2*B*, 3*D*). During the continued application of a constant amount of current the frequency of discharge of any one cell

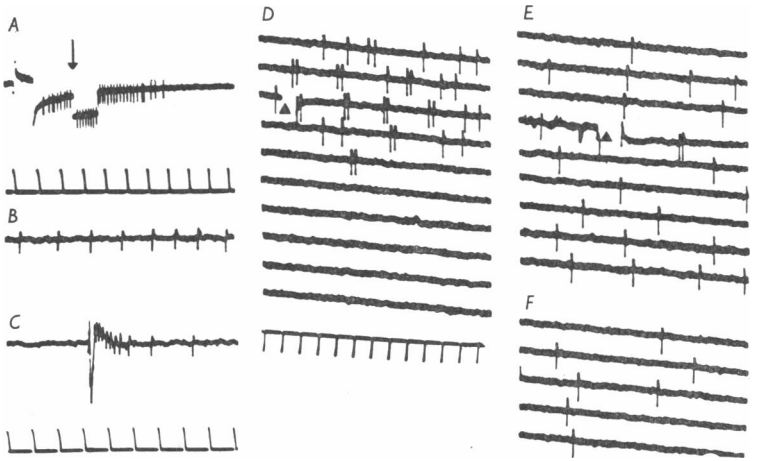


Fig. 3. *A*, discharge evoked by a current of $10 \times 10^{-8}$ A flowing for 90 msec through a barrel containing acetylcholine; the step on the sweep to the right of the arrow has been expanded in *B*. *C*, the same sweep speed as *B* showing the responses evoked by a maximal synaptic stimulus. Time marker, 100 msec for *A*; 10 msec for *B* and *C*. *D*, responses produced 3 sec after the commencement of $15 \times 10^{-9}$ A through the acetylcholine barrel; current ceased at ▲. *E*, responses produced by $100 \times 10^{-9}$ A through the succinylcholine barrel; current ceased at ▲. *F*, 10 sec after *E*. Time marker, 10 msec.

diminished with time. The graph of Fig. 4*A* shows that, when a constant current of $10^{-8}$ A was passed through an electrode containing acetylcholine, the maximum frequency of 49/sec was attained in 1–2 sec and the frequency fell to 18/sec in 1 min. Consequently, when testing the effect of different current strengths or of potentiating or blocking drugs on an individual cell, it was necessary to apply acetylcholine for periods of only 3–4 sec. In addition, interference between doses was avoided by allowing an interval of at least 15 sec between tests. Under these circumstances a maximum frequency was obtained which depended on the rate at which acetylcholine was applied. In Fig. 4*B* are shown two strength-frequency curves obtained at different times from the one cell. In each case the indicated current was passed for 4 sec and

the maximum frequency measured. Similar curves were drawn for several cells and in all cases increase in the rate of application of acetylcholine above a critical value did not give any further increase in frequency. However, this maximum was always less than that obtained when nicotine was used to fire the cell (cf. Fig. 1*B*, *D*), which in turn was less than the maximum frequency obtained when the cell was stimulated synaptically. In Fig. 3*A* a pulse of acetylcholine was used to stimulate a Renshaw cell and the step in the sweep, to the right of the arrow, is expanded in Fig. 3*B*. The frequency of 100/sec was the maximum for this cell even when larger doses of acetylcholine were applied for a longer time. However, with synaptic stimulation (Fig. 3*C*) the initial six responses had a frequency of 700/sec.

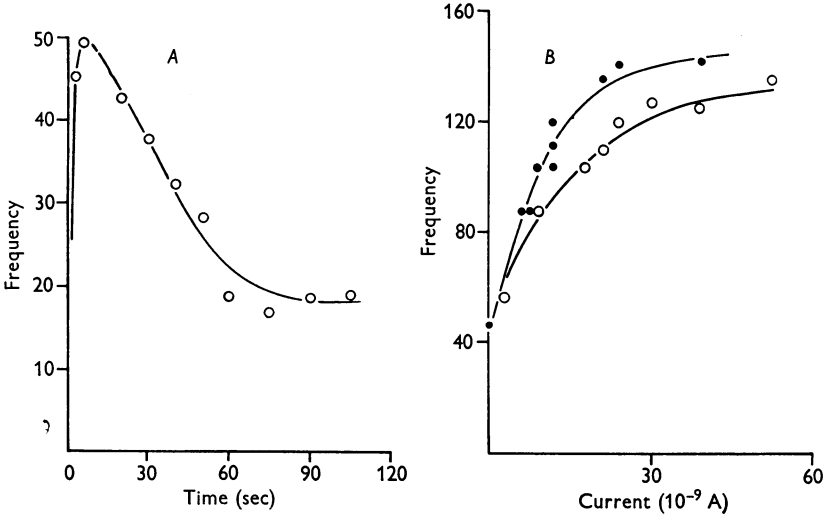


Fig. 4. *A*, frequency of discharge of a Renshaw cell evoked by a constant current of $1{\cdot}25 \times 10^{-8}$ A flowing through a barrel containing acetylcholine, plotted as a function of time. *B*, each point represents the maximum frequency of discharge of a Renshaw cell produced by the indicated current flowing for 3–4 sec through an acetylcholine electrode, there being 15 sec interval between tests. The two series ●, ○ are 10 min apart.

When the acetylcholine current ceased, the cell usually stopped responding within 0·5 sec (Figs. 2*C*, 3*A*, *D*, 5*A*), although several cells showed discharges for 2–3 sec. If a background discharge was initially present, it reappeared after a quiescence of 1–2 sec.

*Substances with an acetylcholine-like action*

When applied by intra-arterial injection, nicotine stimulates Renshaw cells more effectively than does acetylcholine, whereas acetyl-β-methylcholine, carbaminocholine, arecholine and succinylcholine are without effect (Eccles *et al.* 1956). Nicotine when locally applied was also extremely effective in

discharging Renshaw cells. Although the latency usually exceeded that found for acetylcholine, as illustrated in Figs. 5*B* and 6, the maximum frequency of discharge exceeded that obtained using acetylcholine sometimes by a factor of two (cf. Fig. 1*B*, *D*). Further, as also shown in Figs. 5 and 6, when the current through the nicotine-containing barrel ceased to flow, the discharge rate often

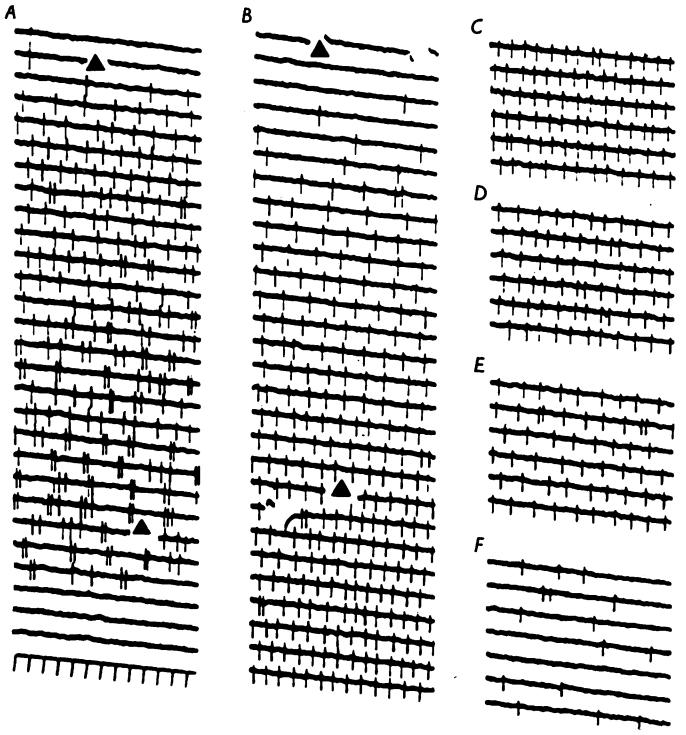


Fig. 5. All records are from the one cell that had almost no background discharge. In *A* a current of $4 \times 10^{-8}$ A flowed through the acetylcholine barrel between the two ▲ points. In *B*, taken 5 min later, the same current flowed through the barrel containing nicotine for a time indicated by the two ▲ points. *C*, *D*, *E* and *F*—5, 10, 15 and 20 sec, respectively, after the second ▲ in *B*. Time marker, 10 msec.

continued to rise to a maximum and then gradually fell over a period of at least 1 min. On this account an extensive investigation into the dose–response relationships could not be carried out; nevertheless, it was clear that the maximum frequency of discharge evoked by nicotine always was less than that produced by synaptic stimulation of the cell.

The other stable acetylcholine-like substances were not very effective when applied in this manner. Succinylcholine produced a low-frequency discharge (Fig. 3*E*), which persisted for minutes after the backing voltage had been restored to the electrode. The responses of Fig. 3*F* were taken 15 sec after this

time, whereas the discharge produced by acetylcholine (Fig. 3*D*) persisted for less than 1 sec when this drug ceased to flow. Similar results were obtained with acetyl-β-methylcholine (Fig. 2*E–H*), carbaminocholine and arecholine. In most cases the latency of the onset of any effect exceeded that found with acetylcholine, the maximum frequency of response was lower than that of acetylcholine and the cessation of firing when current had ceased to flow from the electrode continued as a slow process taking 30–60 sec. In one cell the maximum rate of discharge produced by carbaminocholine equalled that produced by acetylcholine.

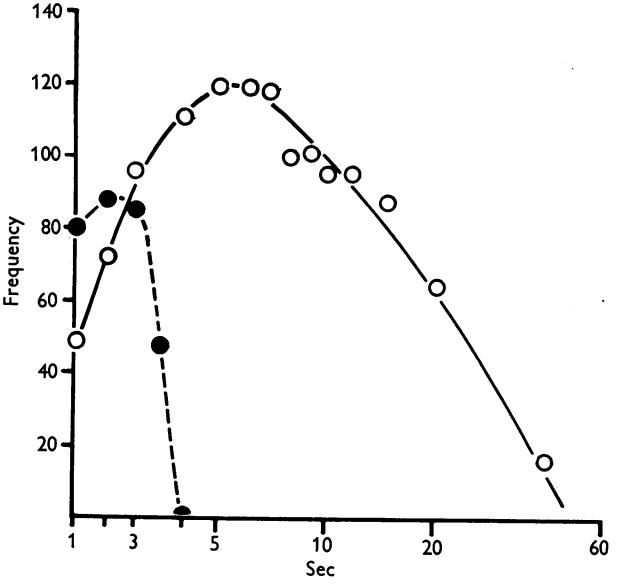


Fig. 6. The frequency of response of a Renshaw cell, measured over intervals of 0·5 sec, and evoked by acetylcholine, ●, and nicotine, ○; semi-log scale. The electrophoretic currents began at zero and ceased at 3 sec. Full series illustrated in Fig. 5.

## DISCUSSION

The difficulties and limitations associated with the electrophoretic application of drugs from glass micro-electrodes have been discussed fully by del Castillo & Katz (1955, 1957*a*). However, a particular difficulty in this present investigation concerns the complete absence not only of visual control of the relationship between the electrode tip and the cell, but also of the ability to orientate the electrode easily with respect to a cell. Although it is possible to make a series of parallel tracks within the spinal cord in the region of a single cell and thereby to improve the recording of its responses, this method is time-consuming and often results in destruction or loss of the unit. Accordingly, once a cell was obtained which responded to stimulation both synaptically and

by acetylcholine, further pharmacological investigation was undertaken. In most cases the extracellular responses were less than 1 mV and it is probable that the cells were 10–40 $\mu$ from the electrode, which offers an explanation of the long latencies found for the actions of some drugs.

The main results of this investigation are concerned with the relative effectiveness with which acetylcholine, nicotine and related substances depolarize a Renshaw cell when applied locally. The fact that all cells tested have responded to acetylcholine, carbaminocholine, succinylcholine and acetyl-$\beta$-methylcholine is in contrast to observations made when these drugs have been administered by injection into the circulation. It is therefore probable that the so-called blood–brain barrier is the principal factor obstructing access to the Renshaw cells (cf. Curtis & Eccles, 1958). It has been demonstrated at the neuromuscular junction (del Castillo & Katz, 1957*b*) that the post-synaptic receptors differ in their sensitivity to drugs of this series. Hence, it would be expected that the effectiveness with which the drugs investigated could discharge Renshaw cells would differ, even if they were applied directly to the post-synaptic region. However, the repetitive response of these cells produced by synaptic activation has suggested that a diffusional barrier, intimately related to the synaptic terminals (Eccles *et al.* 1956), prevents the free diffusion of acetylcholine from its site of liberation and consequently maintains a high concentration for a considerable period. This barrier, functioning to retain acetylcholine, would also be an effective barrier to drugs applied externally. This factor explains the relative inefficiency of the depolarizing agents in discharging Renshaw cells, when their action is compared with the rate at which synaptically released acetylcholine can discharge a single unit. It is unlikely that the low-frequency discharge which acetylcholine evokes when directly applied is a consequence of the activation of a very localized group of post-synaptic receptors. Not only have comparatively large doses of acetylcholine been discharged from the electrode, compared with those used at the neuromuscular junction (del Castillo & Katz, 1957*a*), but the cell size is probably small in comparison to the distance from which the drug is released. Consequently, the actual concentration of acetylcholine would be expected to be virtually uniform around the cell boundaries. By limiting the rate at which electrophoretically applied acetylcholine reaches the post-synaptic receptors, this barrier could also account for the shape of the dose–response curve of Fig. 4*B*.

The relatively short duration of response occurring when acetylcholine ceased to flow from the electrode is presumably due to the local action of cholinesterase both external to and internal to the synaptic barrier. As will be seen in the subsequent paper (Curtis & Eccles, 1958), under the influence of a locally applied anti-cholinesterase the responses evoked by acetylcholine and by synaptic stimulation are greatly increased and prolonged. The responses

evoked by nicotine and the other acetylcholine-like substances are not complicated by enzymatic removal and the time course probably parallels the removal of the drug purely by diffusion. The occurrence of random discharges of Renshaw cells both in single and multiple groups can be ascribed to the leakage of quanta of acetylcholine from the presynaptic terminals, as has been observed at neuromuscular junctions (Fatt & Katz, 1952). This spontaneous discharge can be recorded with single electrodes containing 3 M-KCl or 4 M-NaCl, hence it is not a spurious result due to undetected diffusion from an electrode containing a depolarizing agent. Further, the discharges are not a result of cellular injury, for not only can they be detected at constant frequency by a stationary electrode for several hours, but the frequency often remains unaltered in spite of moving the electrode tip 200–300 $\mu$. The bursts of activity, not unlike that seen with synaptic stimulation, are possibly due to impulses in motor axon collaterals as a consequence of the discharge of the associated motoneurone. However, it is interesting that this type of activity is more common when there is a background of acetylcholine or nicotine, for it is unlikely that it is due to the discharge of motoneurones produced by these drugs. Since very little convergence is necessary to fire Renshaw cells (Eccles *et al.* 1954) the possibility exists that these trains of repetitive responses arise from an axon reflex. It has been demonstrated that a recurrent discharge occurs from muscle when the motor nerve is stimulated (Masland & Wigton, 1940; Eccles, Katz & Kuffler, 1942; Lloyd, 1942) due presumably to the action of released acetylcholine upon the nerve terminals. Consequently, if applied acetylcholine initiates impulses in the terminal branches of the motor-axon collaterals, these impulses, spreading to the associated endings of the same collateral, might give rise to repetitive discharges of a Renshaw cell not unlike those evoked by stimulating the ventral root.

The phenomena of the depression observed in the background discharge following either the response to synaptic stimulation or to applied acetylcholine is probably related to the finding that acetylcholine can desensitize the post-synaptic receptors of muscle (Thesleff, 1955; Katz & Thesleff, 1957). Thus, following a period of repetitive activity due to a relatively high concentration of acetylcholine, the actual sensitivity of the post-synaptic receptors to acetylcholine might become slightly decreased. This may also account for the 'accommodation' observed in Fig. 4*A*, where, under the influence of a constant rate of application of acetylcholine, the frequency of the discharge of the cell diminished with time.

## SUMMARY

1. Methods are described by which drugs can be applied electrophoretically to Renshaw cells located within the ventral horn of lumbar segments of the cat's spinal cord.

2. These cells discharge spontaneously at low frequency. The rate of discharge is increased by acetylcholine, nicotine and related compounds having actions at peripheral cholinergic junctions.

3. There is evidence for the existence of two diffusional boundaries between these cells and substances administered intravenously or intra-arterially.

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