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SOME PROPERTIES AND REFLEX CONNEXIONS OF RESPIRATORY MOTONEURONES OF THE CAT'S THORACIC SPINAL CORD

BY T. A. SEARS*

From The Department of Physiology, Australian National University, Canberra, Australia

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Our understanding of the physiology of limb movements is now firmly based on a comprehensive knowledge of the properties of spinal motoneurones and of the segmental reflexes acting on them. However, our understanding of the physiology of respiratory movements is without such a basis even though, of course, much has been learnt about the supraspinal mechanisms influencing them. Investigations on the latter originate from the work of Legallois (1812) who showed that in the medulla oblongata there is a region 'dans lequel reside le premier mobile de la respiration'. The region accorded such a function is loosely described as the 'respiratory centre'. Alternatively, a 'respiratory centre' is deemed to exist in order both to co-ordinate the activity of respiratory motoneurones at widely differing levels of the neuraxis and to permit the integration of the diverse excitatory and inhibitory pathways influencing respiration (cf. Pitts, 1946). Following Legallois, physiologists have been pre-occupied with the problem of localizing the 'respiratory centre' and in constructing hypotheses as to its function. Consequently, the most important level for the integration of excitatory and inhibitory synaptic actions, the respiratory motoneurone itself, has, with few exceptions (e.g. Ramos & Mendoza, 1959), been largely neglected. On this account alone, the term 'respiratory centre' must be regarded as not particularly meaningful and the author agrees with Liljestrand (1953), that its use should be discarded.

In order to learn something about the integrative function of the respiratory motoneurone, the technique of recording intracellularly from neurones (Brock, Coombs & Eccles, 1952; Woodbury & Patton, 1952) has been applied to respiratory motoneurones of the thoracic spinal cord. The thoracic spinal cord was studied because it offered the opportunity of recording from inspiratory and expiratory motoneurones in the same segment of the spinal cord. In the present paper an account is given of the

* Visiting Wellcome Research Fellow, on leave of absence from the Institute of Neurology, the National Hospital, Queen Square, London, W.C. 1.

general experimental procedures employed and of some of the properties and reflexes of these motoneurones. The paper which follows concerns the nature of the rhythmical changes in membrane potential underlying the periodic excitation, and periodic inhibition, of thoracic motoneurones during spontaneous respiration. A third paper will deal with the properties and distribution of the synapses made directly on thoracic motoneurones by afferent fibres in the intercostal nerves. An account of these findings was presented in July 1963 (Sears, 1964*a*) and briefly in a previous publication (Eccles, Sears & Shealy, 1962). Similar investigations utilizing intracellular recording on phrenic motoneurones have been described by Gill & Kuno (1963*a*, *b*).

METHODS

The experiments were performed on fifty-seven spontaneously breathing cats lightly anaesthetized with sodium pentobarbitone (35 mg/kg body weight; Nembutal, Abbott Laboratories), and on four cats anaesthetized with urethane chloralose mixture (5·6 ml./kg of a solution containing 10 % urethane and 1 % chloralose). Occasionally gallamine triethiodide (flaxedil: American Cyanimid Company) was given and the animal maintained on artificial respiration, with or without bilateral pneumothorax. Supplemental anaesthetic and fluids (0·9 % saline solution or Ringer's solution) were given as required.

Surgical procedures. After venous cannulation and tracheotomy the cat was placed prone, the skin incised from T4 to L1, and the latissimus dorsi muscle cut through bilaterally close to the mid line and sewn back to the skin edges. The semispinalis, longissimus dorsi and iliocostalis muscles were removed bilaterally from T7–11, or more rostrally as required, so giving a clear exposure of the external intercostal muscles extending from the vertebrae to just beyond the angle of the rib. The branches of the posterior rami innervating the interspinalis and multfiidus muscles were freed for stimulation and these muscles were then removed. The greatest obstacle to intracellular recording with the animal breathing spontaneously was the periodic displacement of the spinal cord caused by movements of the underlying vertebrae; those movements were prevented as follows. The laminectomy was limited to three segments, usually from T8 to 10, or other combinations of three. The vertebral column was stretched tautly between flat-jawed clamps gripping the spinous processes of T6 and T7 and pincer clamps gripping the mammillary processes of T11. The vertebrae of T8 and T9 or of T9 and T10 were immobilized by pressing against their lateral processes with screw operated side clamps after opening the capsules of the tubercle articulations. Movements due to venous pulsation were reduced by supporting the pelvis so that the abdomen was pendant and not compressed. Transection of the spinal cord below the level investigated was not necessarily advantageous as this was likely to exaggerate the movements of the spinal cord caused by traction from intercostal nerves of the rostral segments. After all other dissections were completed, and the skin flaps drawn up to form a pool for mineral oil, the dura was opened widely and sometimes it was found advantageous to pass threads through its cut edges to allow the spinal cord to be lifted gently away from the vertebral column.

The external and internal intercostal nerves were freed for stimulation close to the vertebral column as indicated by the white areas on these nerves shown in Fig. 1 of the paper by Eccles *et al.* (1962) and later reproduced when a modification of the nomenclature was introduced (Fig. 1 in Sears, 1964*b*). These nerves and the branch of the posterior ramus were prepared in the three segments on which laminectomy was made. Stimulation of the

external and internal intercostal nerves made it possible to activate antidromically the inspiratory and expiratory motoneurones independently (cf. Sears, 1963), or to assess the reflex effects of stimulation of afferent fibres in the intercostal nerves. All the exposed tissues were washed in Ringer's solution warmed to 39° C which was then drained off and replaced by light mineral oil maintained at 37° C by electric immersion heaters supported in the pool. Electric heaters were also placed beneath the animal so as to help to maintain the rectal temperature between 37 and 38° C.

Recording procedures. The recording electrodes were glass micropipettes filled with solutions of 3M KCl or 2M potassium citrate of resistance 5–20 MΩ. The signals were fed to a cathode follower with capacity neutralization and thence to three amplifiers. These were: a low gain d.c. amplifier connected to a voltmeter and pen recorder used for measuring the membrane potentials; a high gain differential d.c. amplifier having a calibrated switch which could be used quickly to back off the membrane potential so permitting small changes in membrane potential to be recorded at high amplification; an a.c. amplifier (Tektronix Type 122) for measuring synaptic potentials and other evoked activities. The reference electrode was a chlorided-silver plate covered in gauze soaked in saline solution. This plate was earthed through a low resistance across which backing-off potentials (for cancelling out junction potentials, etc.) and calibration voltages were applied. A galvanometer was used to measure currents ($0–50 \times 10^{-9}$A) passed in either direction through the micro-electrode for the purpose of depolarizing or hyperpolarizing impaled cells or, at higher intensities, for clearing the micro-electrode of debris.

Afferent volleys arriving at the dorsal-root entry zone were recorded as cord dorsum potentials through platinum ball electrodes. The diaphragm electromyogram was recorded as previously described (Sears, 1964c).

Stimulation procedures. The nerves were mounted for stimulation on pairs of platinum electrodes and a rotary switch allowed each pair to be selected rapidly for stimulation as soon as a cell was impaled. Stimulation was by capacitor discharges through an isolating transformer and single stimuli or pulsed trains of tetanic stimuli were available.

RESULTS

The general design of the experiments was based on previous investigations from this laboratory. The micro-electrodes were inclined laterally 5–10° and inserted into the spinal cord immediately medial to the dorsal root entry zone through a hole made in the pia. Motoneurones were located by searching for the field potentials of the spikes evoked by antidromic invasion following stimulation of the intercostal nerves. The successful location of motoneurones often required extensive tracking because in the thoracic region the ventral horn is a thin, nearly vertical column not more than 0·5 mm wide. When a cell was impaled, as indicated by the occurrence of a potential of between -40 and -70 mV at the micro-electrode tip, it could be identified as a motoneurone according to whether or not a brief latency spike resulted from stimulation of an intercostal nerve. The presence of an inflexion on the rising phase of the spike showed an intrasomatic rather than axonal location of the micro-electrode (Brock *et al.* 1952; Brock, Coombs & Eccles, 1953; Woodbury & Patton, 1952). By stimulating the external and internal intercostal nerves independently,

it was possible by antidromic invasion to identify impaled motoneurones as inspiratory or expiratory because, in the cat, these nerves innervate respectively the inspiratory and expiratory muscles of the chest and abdominal wall (Sears, 1963, 1964*b*). After recording the spike and its after-potential, reflex actions exerted on the motoneurone by inter-

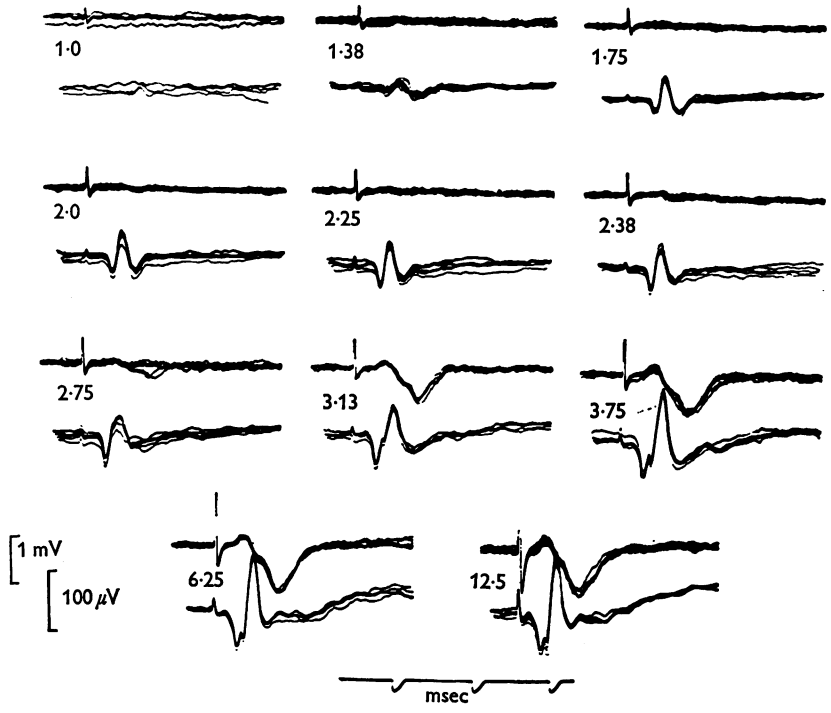

Fig. 1. Antidromic SD-spike potentials evoked by stimulation of the internal intercostal nerve (T 8). Upper trace, recording from micro-electrode in ventral horn (downward deflexion records, unconventionally, negativity of micro-electrode tip). Lower trace, recording of afferent volley recorded at the dorsal root entry zone.

costal afferent fibres were investigated. Continuous d.c. recordings were made on moving film of any spontaneous activity on which the experimentally induced activity was superimposed. Other procedures used are described in the text.

Field potentials. Typical recordings of the antidromic field potentials evoked by stimulating the internal intercostal nerve (T 8) are shown in the upper traces of Fig. 1 (negativity of the electrode is signified, unconventionally, by a downward deflexion). The lower traces show the afferent

volley recorded from the cord dorsum at the root entry zone. No sign of a field potential occurred until the initial or '1a' component (see later) of the afferent volley was nearly maximal at 2·0 times the threshold of the most excitable afferent fibre. With further increases in stimulus intensity the field potential grew incrementally and was nearly maximal at 3·75 times threshold, or at approximately 1·75 times the threshold of the most excitable motor fibre. The exact relation obtained depended on the population of motoneurones sampled by the micro-electrode. Nevertheless, in a given animal the '1a' component was maximal or within 10 or 20% of maximal before a significant number of motor axons was excited. This relation also held for the external intercostal nerve even though its complement of nerve fibres differs from that of the internal intercostal nerve (Sears, 1964*b*). The field potentials of inspiratory and expiratory motoneurones were located at a depth of 2·75–3·5 mm from the cord dorsum but they were not mutually co-extensive. Whereas the expiratory motoneurones were encountered across the full width of the ventral horn, the inspiratory motoneurones showed a definite medial location to a degree which gave a valuable guide as to the position of the micro-electrode tip.

Membrane potentials. In general recording conditions as stable as with lumbar motoneurones were difficult to obtain with thoracic motoneurones, possibly owing to their small size and non-spherical form (unpublished observations; cf. the description of phrenic motoneurones by Keswani, Groat & Hollinshead, 1954; Keswani & Hollinshead, 1956). There were wide variations in the mean membrane potentials of different cells. Such variation was due in part to the chance of obtaining a satisfactory impalement, presumably of the soma, and the subsequent adequate self-sealing of the membrane (cf. Brock *et al.* 1952; Frank & Fuortes, 1955). Although membrane potentials of -70 to -80 mV were obtained in a few cells, the values most commonly recorded were in the range -40 to -65 mV with a mean of about -55 mV, as compared to the 'resting' potential of about -70 mV given by Brock *et al.* (1952). However, these differences almost certainly depend in part on the different functional states of the two types of motoneurones. Unlike the limb motoneurones studied by Brock *et al.* in animals with transected spinal cords, many respiratory motoneurones were discharging repetitively in the anaesthetized animal and others could be evoked into repetitive activity by segmental reflexes. It follows that membrane potentials lower than the 'resting' potential could have been due to synaptically-induced depolarization. It is not without significance that the cells showing the highest membrane potentials did not display the rhythmical modifications of membrane potential, the 'central respiratory drive potentials' described

previously by Eccles *et al.* (1962) and in more detail in the following paper (Sears, 1964*d*).

The antidromic spike. The amplitudes of the antidromic spikes were usually similar to those of the membrane potentials and showed little or no overshoot potential, even in well impaled cells as judged from the presence of a negative after-potential immediately in the wake of the spike

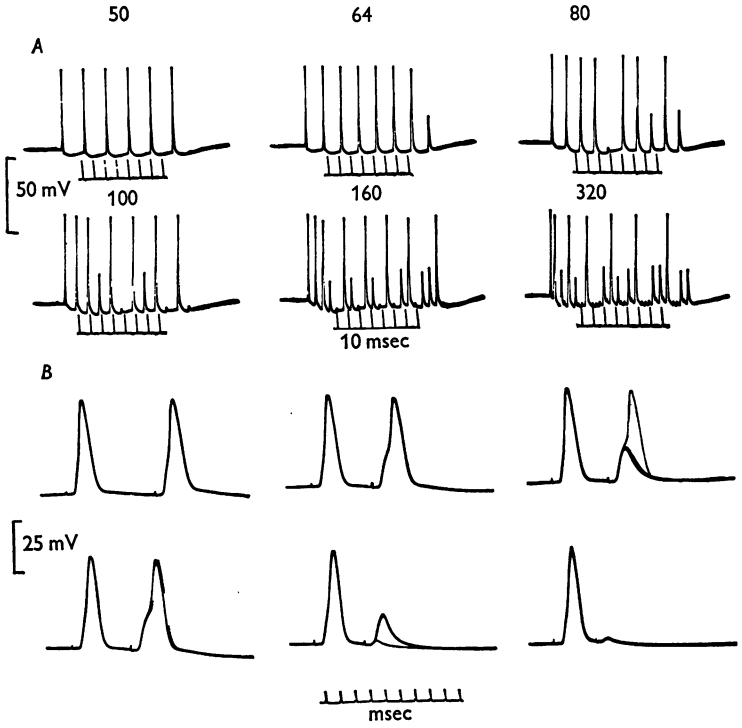

Fig. 2. Repetitive antidromic invasion of a respiratory motoneurone. Intracellular a.c. recording from an inspiratory motoneurone (T8). *A*, stimulation of external intercostal nerve at the indicated frequencies in c/s. *B*, stimulation of external intercostal nerve with closely paired shocks at different intervals.

(cf. Brock *et al.* 1952). In fact, the mean spike amplitude of the group of cells described later (Table 1) was less than the mean membrane potential. A graph showing the relation between membrane potential and spike amplitude of a motoneurone depolarized by an extrinsic current has been published by Eccles (1957, Ch. 2, Fig. 25) and this shows that at low membrane potentials (less than -60 mV) spike amplitude falls below the membrane potential.

By the use of paired shocks to the intercostal nerve, as shown in Fig. 2*B*, the antidromic spike was shown to have the typical double composition

described for lumbo-sacral motoneurones by previous workers (Brock *et al.* 1952, 1953; Woodbury & Patton, 1952; Araki, Otani & Furukawa, 1953; Araki & Otani, 1955; Coombs, Eccles & Fatt, 1955*a*; Frank & Fuortes, 1955; Fuortes, Frank & Becker, 1957; Fatt, 1957; Coombs, Curtis & Eccles, 1957). Thus in the inspiratory motoneurone (T8) of Fig. 2*B*, the inflexion just visible on the rising phase of the first spike was more pronounced in the second spike evoked by a stimulus applied 6·0 msec later (left-hand record of Fig. 2*B*). As the stimulus interval was reduced the inflexion became more pronounced until at 3·7 msec the response alternated between small spikes alone or large spikes, the IS and SD spikes respectively of Coombs *et al.* (1957) or the A and B spikes of Fuortes *et al.* (1957). At 2·8 msec the IS spike also failed and a small M spike was left as the only response to the second stimulus. This M spike is attributed to activity in the most proximal nodes of the medullated axon (Brock *et al.* 1953; Coombs *et al.* 1957). The least or critical interval between stimuli which gave a second SD spike in this motoneurone was 3·7 msec. The mean value was 3·4 msec for thoracic motoneurones which is briefer than the shortest value of 3·8 msec given for lumbosacral motoneurones by Brock *et al.* (1953). Fuortes *et al.* (1957) described a critical interval of 2·6 msec in one particular cell.

The rise time of the SD spike, as measured from the inflexion on the rising phase to the spike summit, was 0·41 msec (S.D. $\pm$ 0·12) in a group of twenty motoneurones with membrane potentials of more than -50 mV. The duration of the declining phase was not measured because of the uncertainty in deciding exactly when the spike terminated.

The dual composition of the spike was also revealed during antidromic invasion evoked at increasing frequencies of stimulation by the failure first of the SD spike then of the IS spike. This differential failure of the spike generating mechanism of the IS and SD membranes is illustrated in Fig. 2*A*. At frequencies of 50 c/s and below both the SD and IS spikes followed each stimulus without intermission, but at higher frequencies there was failure of the SD spike. Unlike the lumbosacral motoneurones studied by Brock *et al.* (1953) the SD spike was generated in thoracic motoneurones at quite high frequencies for three or four impulses without intermission, the discharge only later becoming intermittent to give IS spikes interspersed with M spikes. Table 1 shows the results obtained on a group of thirty cells in which repetitive antidromic invasion was studied over a wide range of frequencies. It may be seen that at 250 c/s, 60 % of the cells were able to give three SD spikes and 44 % of them four SD spikes. At these high frequencies, there was a striking summation of the after-hyperpolarizations following each SD spike, which undoubtedly was in part responsible for the consequent failure of the SD spike. According to

Ito & Oshima (1962) this temporal summation of the spike after-hyperpolarization is associated with an increased potassium conductance of the membrane, so distinguishing it from the mechanism underlying the summation of mammalian C-fibre after-potentials, which has been attributed to the depletion of extracellular potassium (Greengard & Straub, 1958).

TABLE 1. Repetitive antidromic activation of respiratory motoneurones

30 cells examined with frequencies ranging from 80–400 c/s.

Resting potential	55·0 mV S.D. ± 8·8 (30)
Amplitude of spike	50·5 mV S.D. ± 9·8 (21)
Amplitude of after hyperpolarization (AHP)	2·5 mV S.D. ± 1·5 (21)
AHP/Spike	4·9 %
Duration of AHP; range 65–110 msec	

Tetanus frequency	Number of impulses fired: No. of cells firing 2, 3, 4, 5 times			
	2	3	4	5
80	30	26	22	18
100	30	26	24	15
125	30	23	19	17
160	26	21	21	18
200	27	22	20	14
250	24	20	13	8
320	17	9	5	3
400	4	5	3	0

Table 1 shows that the mean amplitudes of the SD spike after-hyperpolarizations were approximately 5 % of the spike amplitudes. The duration of the after-hyperpolarizations ranged from 65 to 110 msec, the mean value of 90 msec being similar to that of the fast flexor muscles of the hind limbs (Eccles, Eccles & Lundberg, 1958; Kuno, 1959). It was not possible to correlate these values with the conduction velocities of the relevant axons because the short conduction distances precluded a reliable estimate of the conduction velocity. However, no correlation was found between the duration of the hyperpolarization and the electrical threshold of the axon expressed relative to the threshold of the nerve. The duration of the after-hyperpolarization was measured from superimposed records using stimulus intensities which just straddled the threshold of the impaled axon so excluding possible contamination by recurrent inhibitory effects (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1958; Kuno, 1959). In fact, with this method no evidence was found of any significant degree of recurrent inhibition. On the other hand, interneurones closely resembling Renshaw cells in their discharge patterns were found among the alpha motoneurones in the ventral horn, but further work is required for their function to be elucidated.

Monosynaptic excitation of thoracic motoneurones by afferent fibres in the intercostal nerve. Stimulation of either the internal or external intercostal

nerves below the threshold of the motor axons, at an intensity adequate to excite only the lowest threshold afferent fibres, evoked in both inspiratory and expiratory motoneurones a brief depolarization (EPSP) of simple time course as illustrated for an expiratory motoneurone (T8) in Fig. 3*C*. The latency of onset of the EPSP, as measured from the summit of the initial positivity of the triphasic action potential recorded at the root entry

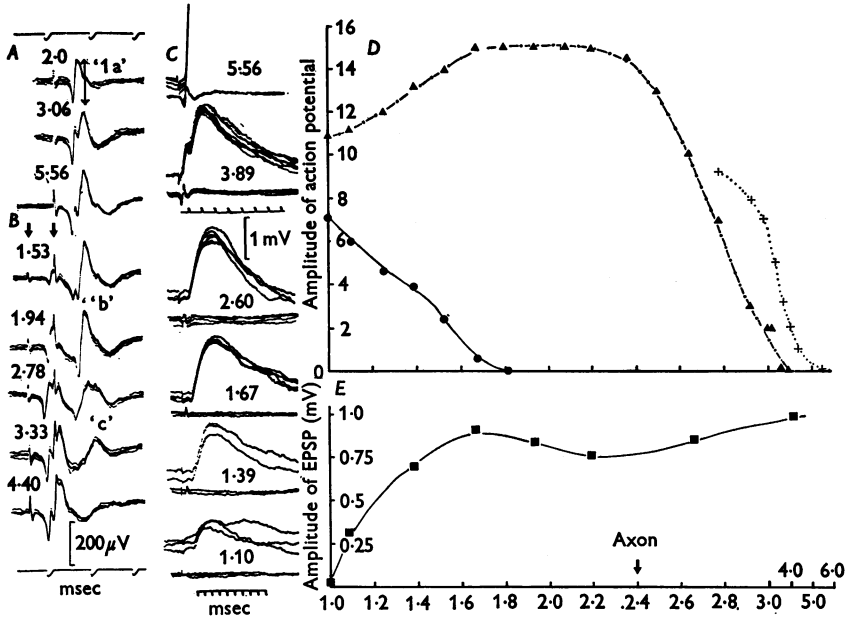

Fig. 3. Correlation of monosynaptic EPSP to '1a' component of afferent volley. *A*, action potential recorded at dorsal root entry zone evoked by stimulation of the internal intercostal nerve (T8) at the indicated stimulus intensities measured relative to threshold. *B*, same as in *A*, but two stimuli (indicated by arrows) 0·6 msec apart. The intensity of the first stimulus was varied as indicated; the second stimulus was kept constant at 5·56 times threshold. The ordinates in *D* show the amplitudes of the three components '1*a*' (filled circles), '*b*' (filled triangles) and '*c*' (crosses) as indicated in *B*, plotted against the intensity of the first shock measured relative to threshold. *C*, Intracellular recording from expiratory motoneurone (T8) in same animal as for *A* and *B*. Monosynaptic EPSPs evoked by stimulation of the internal intercostal nerve at the indicated intensities. In *E*, the amplitudes of the EPSPs are plotted against stimulus intensities relative to threshold.

zone (Fig. 3*A*), was between 0·5 and 0·8 msec in a large number of cells. In twenty-three motoneurones with a mean membrane potential of −55 mV (S.D. ± 10·0), the mean rise time of the EPSP measured on fast sweeps was 1·4 msec (± 0·43). The time for decay to half-maximum ranged from

3·0 to 6·0 msec; occasionally it was considerably longer even when precautions were taken to minimize possible contamination with polysynaptically induced activity by using weak afferent volleys. No essential differences were observed in the EPSPs thus evoked in inspiratory and expiratory motoneurones.

These general features closely resemble those of the monosynaptic EPSPs of lumbosacral motoneurones. According to Eccles (1957; Chapter II, p. 31), with monosynaptic excitatory action, the EPSP can be detected at about 0·5 msec after the primary afferent volley has entered the spinal cord, as has been found in the present investigation. The precise time of onset of the monosynaptic EPSP is determined by the conduction velocity of the relevant fibres, their homogeneity as a group, and their efficacy in depolarizing the post-synaptic membrane to produce a recordable potential. With these qualifications in mind it is concluded that the depolarization described above is a monosynaptically induced EPSP and, accordingly, it will be so described.

The monosynaptic EPSP of thoracic motoneurones shows a further resemblance to that of lumbosacral motoneurones, in that it is evoked by afferent fibres of the lowest electrical threshold. Figure 3*A* shows recordings from the dorsal root entry zone (T8) of the afferent volleys evoked by stimulating the ipsi-segmental internal intercostal nerve (T8) at three different intensities. The compound action potential invariably consisted of at least two components with clearly distinct electrical thresholds. The lowest threshold component will here be described as the '1a' component because of its obvious association with the 1a component of limb-muscle nerves; the inverted commas signify that it arises from nerves which are not wholly muscular in distribution, to which nerves the description 1a strictly pertains. The '1a' component is shown at a stimulus strength 2·0 times threshold, which was slightly supra-maximal for it. The higher threshold 'b' component is shown at a submaximal (3·06 times threshold) and a supra-maximal (5·56 times threshold) intensity. The clear separation of these components was also shown by the use of paired, closely spaced shocks, so timed that the second or test shock fell in the refractory period of the first or conditioning shock. Thus by the action potential that it evoked, the test stimulus revealed the fibre population not excited by the conditioning stimulus. Such a series of records is partially illustrated in Fig. 3*B* and plotted in *D*. When the intensity of the conditioning shock was increased above the threshold of the nerve, the test '1a' component decreased in amplitude and was absent altogether when the test shock was more than 1·8 times threshold. At this intensity of the conditioning stimulus the 'b' component is seen in isolation, and is actually larger since it is no longer superimposed on the opposite

phase of the '1a' component. Further increase of the conditioning stimulus intensity caused the '*b*' wave to diminish and it was completely abolished at about 3·5 times threshold to unmask a still higher threshold component labelled as 'c'. Collected results from sixteen experiments in which paired shocks were used to measure the thresholds and maxima for the two components gave the following figures: The stimulus intensity giving a maximal '1a' response was 1·8T (S.D. $\pm$ 0·37); the stimulus intensity for a threshold 'b' response was 1·87T (S.D. $\pm$ 0·30) and for a maximal 'b' response 3·0T (S.D. $\pm$ 0·78). The mean values conceal the fact that a high stimulus intensity for a maximal '1a' component was correspondingly associated with a high threshold for the 'b' component.

The records of the EPSP shown in Fig. 3*C* were recorded from an expiratory motoneurone of the same segment shortly after taking the records of Fig. 3*A* and *B*. They partially illustrate the plotting in *E* of the relation between stimulus intensity and the amplitude of the EPSP. A comparison of Fig. 3*E* with the curve relating the growth of the '1a' component with increasing stimulus intensity (filled circles in *D*), shows an extremely close correlation. This relation was an invariable finding in approximately 300 motoneurones studied, and applied equally to the growth of the EPSP evoked in inspiratory and expiratory motoneurones by stimulation of either the internal or external intercostal nerves of the same or adjacent segments.

The amplitude of the EPSP was usually maximal, or within 10% of maximum, at an intensity adequate to excite the axon of the impaled motoneurone. If, as in Fig. 3*C* when the stimulus was at 3·89 times threshold intensity, there was failure of the IS, SD spikes so that only the M spike occurred, the EPSP could be recorded at higher stimulus intensities. Records so obtained confirmed that no appreciable increase in amplitude resulted at these higher stimulus intensities. Similarly, when the axon threshold itself was high, the growth amplitude curve of the monosynaptic EPSP in thirty-two expiratory and twelve inspiratory motoneurones was observed to reach a well-defined plateau before the axon was stimulated, as seen in Fig. 4. Further details of the distribution and properties of the monosynaptic EPSP and the distribution of the fibres causing it, will be given in a following paper (Sears, in preparation).

Segmental reflex inhibition of thoracic motoneurones. Stimulation of afferent fibres in the internal intercostal nerves may inhibit thoracic respiratory motoneurones as illustrated in the records from an expiratory motoneurone shown in Fig. 4. In *B*, the amplitudes of the monosynaptic EPSPs of the superimposed records shown in *A*, taken from an expiratory motoneurone, are plotted as ordinates, against the respective stimulus intensities relative to threshold which evoked them as abscissae. The

EPSP reached a maximum amplitude with a stimulus about 1·3 times threshold and remained roughly constant until 1·6 times threshold. This motoneurone was not discharging spontaneously but it showed a 'central respiratory drive potential' or CRDP, as may be seen by the inconstant

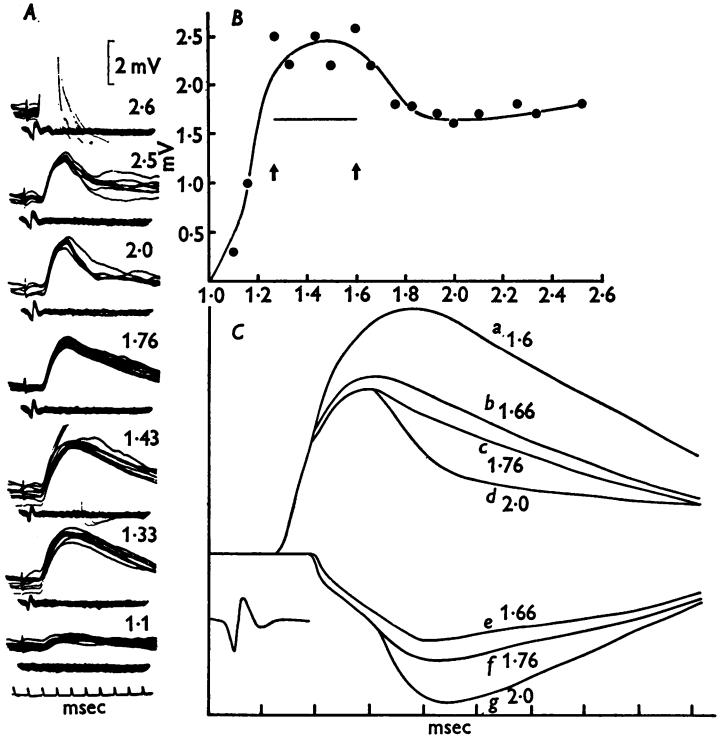

Fig. 4. Segmental reflex inhibition of a respiratory motoneurone. The upper traces in *A* show intracellular a.c. recordings from an expiratory motoneurone (T9) evoked by stimulation of the internal intercostal nerve at the indicated stimulus intensities measured relative to threshold. Note, at 1·43 times threshold, reflex discharge in some sweeps. *B*, plotting of peak amplitude of the EPSP against stimulus intensity measured relative to nerve threshold. Horizontal line indicates range of stimulus intensities at which reflex discharge occurred. *C*, *a*, *b*, *c* and *d* tracings of the records at the indicated stimulus intensities; *e*, *f* and *g* obtained by graphical subtraction of the curves *b*, *c* and *d* respectively, from curve *a*.

level of the base line in some of the records. The exact level of the membrane potential cannot be determined from these records which were made through an a.c. amplifier, but the rapid phase of the CRDP was not completely attenuated with the coupling time constant in use (T.C. 200 msec). Because of summation of the EPSP with the depolarizing phase of the CRDP (Eccles *et al.* 1962; Sears, 1964*d*) orthodromic reflex firing

occurred during each expiration as seen in about half the superimposed sweeps taken at 1·43 times threshold. In the remainder of the sweeps it may be assumed that the EPSPs were evoked before the cell was maximally depolarized by the CRDP. This monosynaptically induced firing during expiration occurred with the entire range of stimuli between 1·3 and 1·6 times threshold, as indicated between the two arrows in *B*. However, with stimulus intensities greater than 1·6 times threshold, the reflex firing ceased, owing to a reduction in the peak amplitude of the EPSP. In Fig. 4*C*, the tracings *a*, *b*, *c* and *d* were made from the EPSPs evoked at the indicated stimulus intensities. Tracings *e*, *f* and *g* show the difference obtained graphically by subtracting curves *b*, *c* and *d* from the curve *a*, the assumption being that the curve so obtained represents the time course of an inhibitory synaptic potential (IPSP) added to the response by the excitation of higher threshold afferent fibres. No account has been taken of the enhancement of the IPSP by the concurrent depolarization due to the EPSP in this analysis (Coombs, Eccles & Fatt, 1955*c*; Curtis & Eccles, 1959). The inhibitory curve shows two phases; an early phase which prevents the EPSP from reaching its peak amplitude and is caused by relatively low-threshold fibres (approximately 1·66 times threshold in this cat); and a later phase, which is more prominent at the higher stimulus intensities, that is responsible for the late concavity of the EPSP. The onset of this early inhibition occurs within 0·6–0·8 msec of the onset of the EPSP. Early inhibition, cutting off orthodromic spikes reflexly evoked at lower stimulus intensities, was observed in five other expiratory cells. In one of them, the reflex discharge was also evoked by the EPSP which resulted from stimulation of the adjacent internal intercostal nerve, and this discharge was similarly abolished when the stimulus intensity was increased. A further twelve expiratory motoneurones, which showed no reflex discharges, nevertheless, showed a diminution in amplitude of their monosynaptic EPSPs at higher stimulus intensities. The early inhibition appeared to develop at stimulus intensities below those which excited a significant fraction of the motor axons but above that producing a maximal EPSP. The threshold of the axon of the cell in Fig. 4 was itself fairly high, but the mean threshold of eleven other motoneurones impaled in the same segment was 2·0 times, and none was below 1·8 times threshold of the '1a' axons.

In six expiratory motoneurones, an IPSP occurred with stimuli supramaximal for the '1a' component applied to the adjacent internal intercostal nerve. The shortest latency of such inhibitory potentials was 0·8 msec longer than the latency of the EPSP evoked in other expiratory motoneurones by a weaker stimulus (maximal for '1a'), applied to the same nerve. On no occasion was inhibitory action observed from the same

or adjacent intercostal nerves with stimulus intensities below those that evoked a maximal '1a' component in the cord dorsum potential, or a maximal EPSP in the impaled motoneurone.

DISCUSSION

In general, the antidromically evoked SD spikes of the thoracic respiratory motoneurones followed considerably higher frequencies of stimulation than did those of the lumbosacral motoneurones described by Brock *et al.* (1953) which failed altogether at about 42 c/s. Similarly the mean critical interval of 3·4 msec for a second SD spike to paired stimuli was shorter than for lumbosacral motoneurones but not so brief as reported for phrenic motoneurones (0·2 msec ± 0·7 S.D.) by Gill & Kuno (1963*a*) or for Betz cells of the cat's motor cortex (Phillips, 1959) in which IS SD transmission rarely failed. This higher safety factor for the transmission of the impulse from the axon and initial segment, to the soma-dendritic membrane (cf. Gill & Kuno, 1963*a*), is unlikely to be due to the lower membrane potential of the thoracic motoneurones because a decline in membrane potential was found to impair the generation of SD spikes, presumably by cathodal depression (cf. Coombs, Eccles & Fatt, 1955*b*). Nor may it be attributed *per se* to the short duration of the after-hyperpolarization because the values obtained were identical to those of the flexor motoneurones, which did not follow high frequencies, as described by Eccles *et al.* (1958) and Kuno (1959). Furthermore, it is doubtful whether any concomitantly evoked depolarization caused by summation of successive monosynaptic EPSPs (Sears, unpublished observations) was assisting antidromic invasion because summation of the after-hyperpolarizations (Brock *et al.* 1953; Ito & Oshima, 1962) dominated the response (Fig. 2*A*). The absence of a significant degree of recurrent inhibition, as also described for phrenic motoneurones by Gill & Kuno (1963*a*), might contribute to the higher safety factor for SD transmission, but not completely because the duration of this inhibition is about 40 msec, at least to single stimuli (Eccles *et al.* 1954), so that the duration of the after-hyperpolarization would have been a more significant factor in limiting the discharge rate. One other possibility is that the morphology of the thoracic motoneurones may favour antidromic SD invasion but no quantitative data is yet available for this possibility to be considered in detail.

It would, of course, be a teleological advantage for respiratory motoneurones not to be limited in their discharge rates. The maximal rate of orthodromic discharge for thoracic alpha respiratory motoneurones is not known for certainty, since the possibility now arises that the high discharge rates in what were thought to be intercostal alpha motoneurones during

asphyxia, described by Bronk & Ferguson (1934) may actually have been those of fusimotor neurones (cf. Sears, 1963).

The relatively short duration of the after-hyperpolarizations and their lack of correlation with axonal threshold is of interest in relation to the work of Andersen & Sears (1964). They have shown that cat intercostal muscles comprise both fast and slow motor units but the axons innervating them could not be distinguished on the basis of their electrical thresholds as would be predicted from investigations on fast and slow limb muscles (Eccles *et al.* 1958). Evidently, respiratory motoneurones of the thoracic spinal cord show specific properties which distinguish them from those of the lumbosacral spinal cord and, in this respect, they resemble the phrenic motoneurones (Gill & Kuno, 1963*a*).

The cat intercostal muscles contain many muscle spindles (Huber, 1902) but the monosynaptic excitation of their motoneurones has not previously been described. The results of earlier work has revealed only their activation by polysynaptic pathways (Downman, 1953, 1955; Sumi, 1963) perhaps owing to the mixed inhibitory and excitatory action exerted by all but the weakest afferent volleys.

The monosynaptic EPSP was shown to correlate closely with the excitation in the external and internal intercostal nerves of the lowest threshold afferent fibres giving the '1a' component of the afferent volley recorded at the root entry zone. The 1a component of limb-muscle nerves is known to arise almost exclusively from the primary endings of the muscle spindles (Hunt, 1952, 1954). These fibres convey monosynaptic excitation to motoneurones of the same and synergic muscles (Lloyd, 1943*a*, *b*) and they are responsible for 90–100% of the monosynaptic EPSP of motoneurones of leg muscles (Eccles, Eccles & Lundberg, 1957).

Because the external intercostal nerve is a pure muscle nerve, and because histological work shows that the largest afferent fibres in the internal intercostal nerve are distributed to its muscular branches (Sears, 1964*b*), it is concluded that the '1a' component corresponds to the 1a component of the limb muscle nerves. A further similarity between the '1a' and 1a components is the distribution of monosynaptic excitation by '1a' afferents to the synergic motoneurones of the adjacent segments (Eccles *et al.* 1962).

By analogy with the 'direct inhibition' exerted by the 1a component of the group 1 muscle nerve volley (Lloyd, 1946; Laporte & Lloyd, 1952; Bradley, Easton & Eccles, 1953; Bradley & Eccles, 1953; Eccles, Fatt & Landgren, 1956; Eccles *et al.* 1957) it might have been expected that '1a' afferents from expiratory muscles would, by 'direct inhibition', inhibit their physiological antagonists the inspiratory motoneurones, and vice versa. No such action was observed. Inhibitory action was evoked in

expiratory motoneurones but only by fibres of higher electrical threshold than those giving the '1a' component; it was not established whether these came from muscle or skin, or both.

SUMMARY

1. Intracellular recordings were made from neurones of the cat's thoracic spinal cord. Antidromic invasion from the external or internal intercostal nerves respectively, identified impaled cells as inspiratory and expiratory motoneurones.

2. The antidromic spike resembled that of lumbosacral motoneurones and showed an inflexion on its rising phase signifying a two-stage invasion, first of the initial segment (IS spike) and then of the soma-dendritic regions (SD spike). The critical interval at which the SD spike to a second shock failed was 3·4 msec. The SD antidromic spike followed higher frequencies of repetition than in lumbosacral motoneurones.

3. Monosynaptic excitatory post-synaptic potentials were evoked in inspiratory and expiratory motoneurones by excitation of the lowest threshold afferent fibres in the intercostal nerves. These fibres are responsible for the initial or '1a' component of the afferent volley recorded at the dorsal root entry zone.

4. No inhibitory actions were observed of the fibres giving rise to the '1a' component. Stimuli supra-maximal for the '1a' component excited fibres which exerted an inhibitory action on expiratory motoneurones.

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