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REFLEX CONNEXIONS OF MOTONEURONES OF MUSCLES INVOLVED IN HEAD MOVEMENT IN THE CAT

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SUMMARY

1. The reflex connexions from muscle afferents and ventral root fibres to the motoneurones of the muscles biventer-cervicis, complexus, sternocleidomastoid, trapezius and splenius, the principal muscles involved in head movement in the cat, were studied with the technique of intracellular recording.

2. Electrical stimulation of homonymous muscle afferents of biventer-cervicis and complexus, sternocleidomastoid and trapezius, at strengths below 1·6 times threshold of the dorsal root afferent volley, produced monosynaptic e.p.s.p.s in the corresponding motoneurones. Recruitment of higher threshold muscle afferents produced additional p.s.p.s with longer central delays.

3. Stimulation of low-threshold muscle afferents did not produce any p.s.p.s in the motoneurones of the ipsilateral antagonist. Stimulation of higher threshold afferents evoked i.p.s.p.s with central delays longer than 1·6 msec, or mixed e.p.s.p.-i.p.s.p.s in the ipsilateral antagonist.

4. Mixed e.p.s.p.-i.p.s.p.s or i.p.s.p.s with central delays longer than 1·5 msec were evoked in trapezius motoneurones upon stimulation of high threshold afferents from biventer-cervicis and complexus, while stimulation of low-threshold biventer-cervicis and complexus afferents evoked no p.s.p.s in trapezius motoneurones.

5. Stimulation of contralateral low-threshold biventer-cervicis and complexus afferents evoked a sequence of i.p.s.p. disinhibition in sternocleidomastoid motoneurones, and vice versa, with central delays longer than 1·7 msec.

6. Stimulation of the deafferented biventer-cervicis, complexus, splenius, sternocleidomastoid and trapezius muscle nerves frequently activated interneurones in the ventral horn at monosynaptic central delays. Activation of homoynmous ventral root fibres rarely evoked p.s.p.s in biventer-cervicis, complexus, splenius or sternocleidomastoid motoneurones, while it produced disynaptic i.p.s.p.s in 80 % of trapezius motoneurones.

7. It is concluded that Ia reciprocal inhibition and recurrent inhibition, two reflex circuits which are so prominent in limb segments of the spinal cord, do not play a major role in the generation of head movement. Rather, head movement may be primarily controlled from supraspinal centres.

INTRODUCTION

The synaptic effects of descending tracts at the cervical and lumbar enlargements of the cat spinal cord are primarily mediated through interneurones which are

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involved in segmental reflex pathways (Lundberg, 1975). In contrast, motoneurones of muscles involved in head movement receive monosynaptic projections from many descending tracts (Wilson & Yoshida, 1968; Peterson, Pitts & Fukushima, 1978; Fukushima, Pitts & Peterson, 1978; K. Fukushima, N. Hirai and S. Rapoport, unpublished observations). These direct descending inputs to collic motoneurones have been described in detail, while little attention has been given to the segmental reflex connexions involving these motoneurones (Anderson, 1977). The present investigations were conducted in order to elucidate reflex pathways originating from low-threshold muscle afferents and ventral root fibres to homonymous, heteronymous and antagonist motoneurones of collic muscles.

Electrophysiological studies in th lumbo-sacral segments of the spinal cord have revealed that α-motoneurones receive excitatory monosynaptic connexions from Ia afferents of their own and synergistic muscles (Lloyd, 1946; Mendell & Henneman, 1971). This finding has been extended to include motoneurones in other regions of the spinal cord (Schmidt & Willis, 1963; Sears, 1964). The motoneurones of muscles which extend the head also receive monosynaptic e.p.s.p.s from homonymous and heteronymous muscle afferents (Wilson & Maeda, 1974; Anderson, 1977). However, it has been suggested that spindle primaries in collic muscles may not consist of a homogeneous population of group I fibres; rather, they may be composed of fibres having a wide diameter spectrum (Richmond, Anstee, Sherwin & Abrahams, 1976). Thus, it is as yet unresolved whether the monosynaptic e.p.s.p. of collic motoneurones, like the Ia e.p.s.p. in the limbs, is produced by a homogeneous group of low-threshold muscle afferents.

Antagonistic flexor and extensor muscles in the hind limb are reflexly linked by reciprocal Ia inhibition (Lloyd, 1941, 1946). However, Ia reciprocal inhibition is not a universal reflex arrangement between antagonists; it appears to be a specialization which has evolved for reflex control of particular motor functions (R. M. Eccles & Lundberg, 1958; Sears, 1964; Kidokoro, Kubota, Shuto & Sumino, 1968). At present, it is not known whether reciprocal Ia inhibition plays a role in the control of head movement. Head flexion is effected by the muscle sternocleidomastoid, and extension by the synergistic muscles biventer-cervicis and complexus. The reflex organization between the motoneurones of these two antagonistic muscles groups has been investigated in order to determine whether reciprocal Ia inhibition is a prominent organizational arrangement among collic muscles.

Recurrent inhibition is a salient feature of spinal circuits involving limb motoneurones (Eccles, Eccles, Iggo & Ito, 1961; Thomas & Wilson, 1967). However, like Ia reciprocal inhibition, it is not present in all spinal circuits (Gill & Kuno, 1963; Sears, 1964; Mackel, 1978). In order to determine whether recurrent inhibition is involved in the reflex control of collic motoneurones, the synaptic inputs from ventral root fibres to these motoneurones have been studied.

Some of the observations presented in this paper have been communicated at a meeting (Rapoport, 1977).

METHODS

These experiments were performed in cats weighing 2·5–4·0 kg. Three different anaesthetic protocols were utilized. One group of cats was sedated with ketamine (Vetalar, Parke-Davis), and then anaesthetized with a percutaneous, I.V. injection of chloralose, 50 mg/kg, with additional doses as required during the course of the experiment. A second group of cats was anaes-

thetized with sodium pentobarbitone (Nembutal, Abbott) 30–35 mg/kg I.P., with additional I.V. doses as required during the course of the experiment. One experiment was performed in a precollicularly decerebrated, unanaesthetized cat. All surgery before decerebration was performed under an anaesthetic mixture of halothane (Fluothane, Ayerst), nitrous oxide, and oxygen. Gas anaesthesia was discontinued 30 min after decerebration, and 4 hr before any recording.

Blood pressure was constantly monitored from the femoral artery, and when necessary maintained above 80 mmHg by intravenous administration of normal saline, dextran, or with an infusion of metaraminol bitartrate (Aramine, Merck). All animals were paralysed with gallamine (Flaxedil, Davis and Geck), and artificially respirated during the recording session. Bilateral pneumothorax was produced before recording. The temperature of the cats was monitored with a rectal thermometer, and maintained between 36–38 °C by a radiant heat lamp. At times the temperature of the oil pool was also monitored. This was always found to be in the range of 36–38 °C when the rectal temperature was in a similar range.

The nerves to sternocleidomastoid, trapezius, the C2 and C3 ventral rami which carry muscle afferents from sternocleidomastoid and trapezius, and the C2 and C3 branches of the nerves to biventer-cervicis and complexus were dissected and mounted on bipolar platinum-wire electrodes for stimulation. In some experiments the nerves to splenius, the contralateral C2 and C3 branches to biventer and complexus, and the C2 and C3 ventral rami were also dissected and mounted for stimulation. (The crossed experiments were all performed on chloralose-anaesthetized cats.) In the experiments on recurrent inhibition each of the nerves dissected was divided into two branches, and each branch was mounted on a separate electrode for independent stimulation. All of these experiments were conducted on cats anaesthetized with sodium pentobarbitone. In several experiments the vestibular nerve was stimulated bipolarly. This was accomplished by implanting a silver-wire electrode, insulated except at the spherical tip, on the whole vestibular nerve. An identical indifferent electrode was positioned on nearby bone. In segmental reflex experiments a laminectomy was performed to expose the C1–C3 segments of the spinal cord. A small patch of dura and the underlying pia, overlying the recording area, was removed, with great care taken to avoid damage to any spinal blood vessels. In the experiments on recurrent inhibition, a laminectomy was performed from C1 to C7, and the C1–C7 or C8 dorsal roots were cut, intradurally, on the side ipsilateral to stimulation and recording. The exposed spinal cord and nerves were covered by a pool of mineral oil.

Stimuli were delivered to the nerves by either a constant current or a constant voltage stimulator. Stimuli consisted of rectangular pulses of 0·1 msec duration, delivered at a rate of 4/sec. Intracellular recordings were obtained with glass micropipettes filled with 2 M-potassium acetate, having tip resistances of 2–4 MΩ, and tip diameters of 1·5 μM. Conventional circuits were used for recording and passing currents through the pipettes. Dorsal root volleys were monitored by a ball electrode which was positioned at the dorsal root entry zone of the segment being stimulated. Multiple shock stimuli and injection of polarizing current were routinely applied, in order to enhance post-synaptic effects which were not apparent at the resting membrane potential. All motoneurones were identified by antidromic invasion of the appropriate muscle nerves.

Data analysis

The DC output of the amplifier used for intracellular recording was fed to a PDP-11-45 computer programmed to perform on-line averaging. Data were stored by photographing the amplified intracellular records from a CRT, or by storing the averaged computer records on magnetic tape. For analysis of p.s.p.s the intracellular record and the juxtacellular record were superimposed, and their point of departure taken as the onset of the p.s.p. Central delays were measured from the positive peak of the dorsal root volley. The latencies of recurrent effects were measured from the positive peak of the field potential produced by the antidromic activation of motoneurones (Eccles, Fatt & Koketsu, 1954).

RESULTS

Monosynaptic e.p.s.p.s from low threshold afferents

It has already been shown by Wilson & Maeda (1974) and Anderson (1977) that monosynaptic e.p.s.p.s are produced in biventer, complexus and splenius motoneurones upon stimulating those homonymous afferents which enter the spinal cord

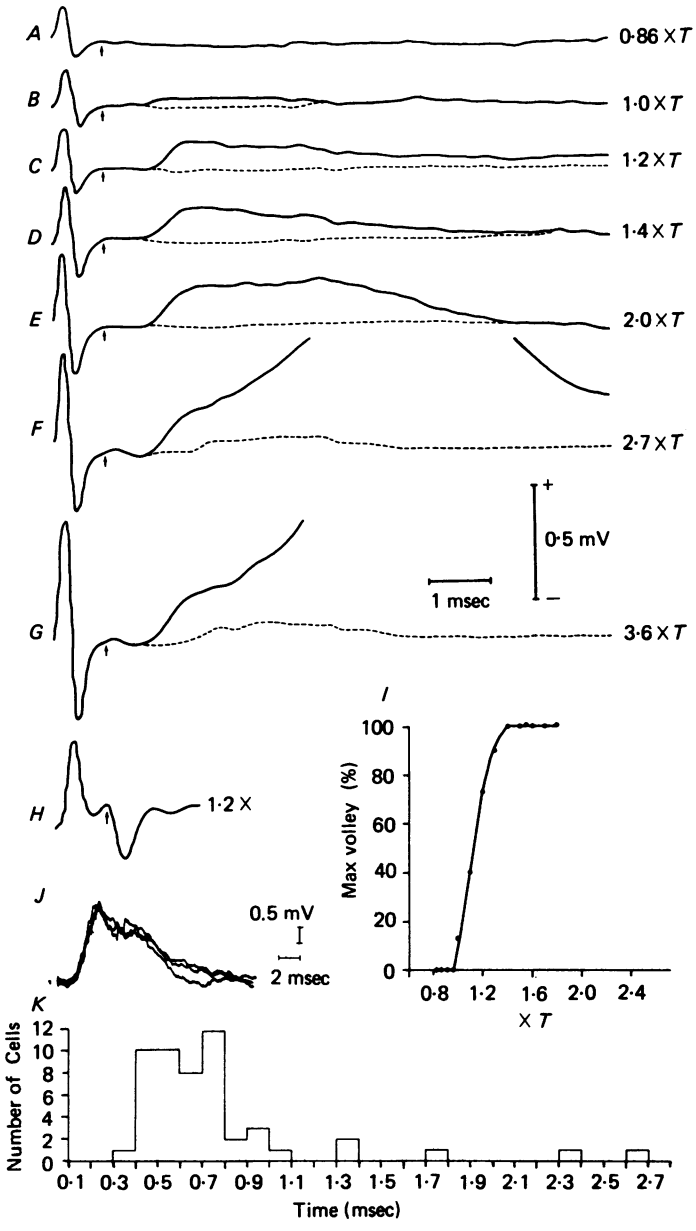

Fig. 1. E.p.s.p. elicited in a sternocleidomastoid motoneurone upon stimulation of homonymous afferents (C2 ventral ramus). *A–G*, intracellular records obtained as the afferents were stimulated with progressively stronger shocks. Stimulus intensities, in multiples of threshold, are indicated on the left of each tracing. Dotted lines depict the juxtacellular fields. *H*, afferent volley recorded at the dorsal root entry zone. First positivity in all tracings is the stimulus artifact. Upward arrow indicates the positive peak of the afferent volley. Central delay of e.p.s.p. was measured from the positive peak of the afferent volley to the point of divergence of intracellular and juxtacellular records. *I*, size of the afferent volley (peak to peak) as percentage of its maximum size, plotted as a function of stimulus strength. *J*, oscillographic record of an e.p.s.p. in another sternocleidomastoid motoneurone, obtained upon stimulation of C2 ventral ramus. Three superimposed sweeps, stimulus strength $2{\cdot}5 \times T$. *K*, segmental delays of earliest e.p.s.p.s produced in fifty-two collic motoneurones upon stimulation of the homonymous low-threshold afferents. (*A–H* are tracings from fifty sweep computer averaged records.)

via the spinal nerves of the same segment as the motoneurones studied. However, it remains to be established whether these monosynaptic e.p.s.p.s are produced solely by the lowest threshold muscle afferents, as are the Ia e.p.s.p.s in the lumbo-sacral spinal cord.

Stimulus strengths required to produce the e.p.s.p.s. The threshold of the earliest e.p.s.p. produced in a trapezius, sternocleidomastoid, or biventer-complexus motoneurone upon stimulating the homonymous muscle afferents (Fig. 1*A*, *B*, *I*) is equal to the threshold of the earliest component of the afferent volley at the dorsal root entry zone. As the stimulus strength is increased beyond threshold, the amplitude of the early e.p.s.p. increases, reaching a maximum at a low stimulus strength. Most of the growth in amplitude of the early e.p.s.p. occurs at stimulus strengths below 1·6 times threshold (Fig. 1*A–G*). The amplitude of the afferent volley usually reaches a maximum, and plateaus at a stimulus strength 1·4 times its own threshold (Fig. 1, *I*). As the stimulus strength is increased beyond 1·6 times threshold the afferent volley broadens and its peak occurs later, a finding which is indicative of recruitment of higher threshold fibres. Of the fifty-two motoneurones studied for the presence of monosynaptic e.p.s.p.s, two biventer-complexus, five sternocleidomastoid and three trapezius motoneurones were subjected to the detailed e.p.s.p. amplitude versus stimulus strength analysis which is illustrated in Fig. 1, and similar results were obtained in all ten cases. The stimulus strengths (in multiples of threshold of the earliest component of the afferent volley) necessary to elicit these early e.p.s.p.s, and the behaviour of their amplitudes as a function of stimulus strength, are similar to these same properties of the Ia e.p.s.p. observed in the hind limb (see for instance Eccles, Eccles & Lundberg, 1957). The amplitudes of the earliest homonymous e.p.s.p.s ranged from 0·1 to 2·0 mV (mean = 0·4, n = 52), in motoneurones with resting potentials of 35–75 mV.

Stimulation of the homonymous muscle nerves at strengths higher than 1·6 times threshold gives rise to additional p.s.p.s which occur with longer central delays. These later p.s.p.s continue to grow in amplitude as the stimulus strength is increased (Fig. 1*E-G*). In some instances these later, higher threshold p.s.p.s have been hyperpolarizing in nature (seven cases in biventer and complexus, thirteen in trapezius, five in sternocleiodomastoid).

Latency. The distributions of central delays of the early, low-threshold e.p.s.p.s were very similar in the three motoneuronal types studied, and are summarized in Fig. 1*K*. The earliest e.p.s.p. observed occurred at a central delay of 0·3 msec. The median central delay of the early e.p.s.p.s was 0·7 msec. These values fall within the monosynaptic range, and resemble those obtained for the Ia e.p.s.p. in the lumbar cord (Eccles *et al.*, 1957). In five instances in which no monosynaptic, low-threshold effect was seen, a polysynaptic, low-threshold effect was observed (Fig. 1*K*).

Effects from homonymous afferents located in segments different from the motoneurone studied. The muscles sternocleidomastoid, trapezius, biventer and complexus are each innervated by rami of spinal nerves arising from multiple spinal segments (Corbin & Harrison, 1938; Richmond & Abrahams, 1975). Stimulation of afferents, from these muscles, which enter the spinal cord at two different segments produce monosynaptic, low threshold e.p.s.p.s in all homonymous motoneurones in which this phenomenon was examined. The latency distributions of effects from the C2

and C3 segments in the same motoneurones are very similar. It thus appears likely that each motoneurone culls afferent input from many of the afferents from several of the spinal segments which innervate its muscle. A situation not unlike that which obtains in the lumbar segments (Mendell & Henneman, 1971).

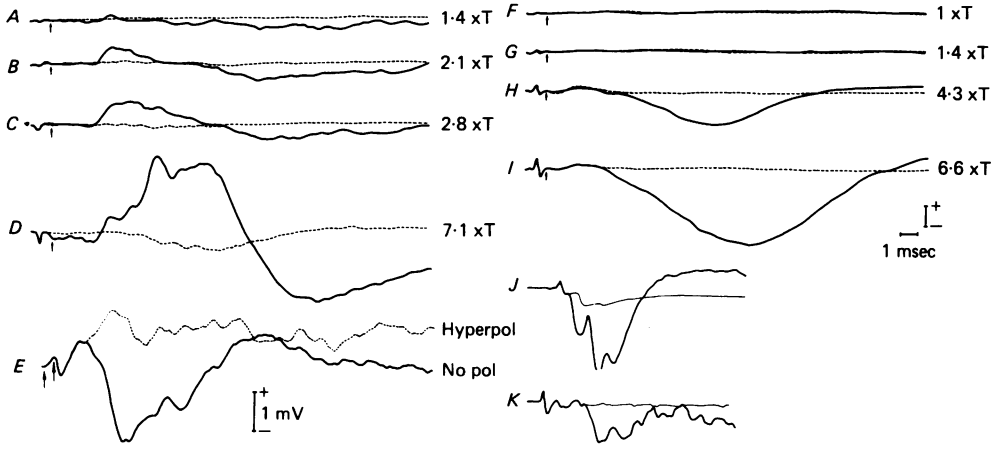

Fig. 2. *A–D*, e.p.s.p.–i.p.s.p. sequence obtained in a sternocleidomastoid motoneurone upon stimulation of the C3 biventer and complexus nerve. Continuous line depicts the intracellular record, dotted line the juxtacellular field. Stimulus intensity, in multiples of threshold of the lowest threshold fibres, is indicated to the left of each trace. Upward arrow marks the location of the positive peak of the afferent volley recorded at the dorsal root entry zone. Stimulus artifact depicted by initial negativity. *E*, i.p.s.p. recorded in a biventer and complexus motoneurone upon stimulation of the ipsilateral C2 ventral ramus at 8 times threshold. Interrupted line depicts partial reversal of i.p.s.p. by injection of 20 nA of hyperpolarizing current through the recording electrode. First arrow: stimulus artifact. Second arrow: latency of positive peak of afferent volley recorded at dorsal root entry zone. The locally recorded afferent volley is seen on this trace as the first positive-negative deflexion. Note separate voltage calibration for this trace. *F–I*, intracellular records obtained in a sternocleidomastoid motoneurone upon stimulation of the ipsilateral C2 biventer and complexus nerve, as the stimulus strength is progressively increased. Stimulus strength, in multiples of threshold for the lowest threshold fibres, is indicated to the right of each trace. Interrupted line depicts the juxtacellular field. Upward arrows mark the positive peak of the afferent volley at the dorsal root entry zone. Initial positive-negative deflexion is the stimulus artifact. *J*, i.p.s.p. evoked in a C2 biventer and complexus motoneurone upon vestibular nerve stimulation. First positivity is the locally recorded vestibulospinal volley. *K*, i.p.s.p. evoked, in same motoneurone as *I*, by stimulation of ipsilateral C2 ventral ramus. First positivity is the stimulus artifact, the second positivity is the locally recorded afferent volley. Thin continuous lines in *J–K* depict the juxtacellular fields. Voltage calibrations: *A–D*, 0·125 mV; *F–I*, 0·5 mV; *J*, 0·25 mV; *K*, 0·1 mV.

Ipsilateral interactions between spinal accessory and biventer cervicis-complexus

The purpose of the experiments summarized in this section is to recount whether any evidence could be gained for Ia reciprocal inhibition between the spinal accessory motoneurones (sternocleidomastoid and trapezius) and the motoneurones of biventer-cervicis and complexus.

Stimulation of the nerves to biventer and complexus, at strengths which activated only the lowest threshold afferents (below $1{\cdot}6 \times T$), produced no p.s.p.s in sterno-

cleidomastoid (twelve motoneurones tested, Fig. 2*A*, *F*, *G*). Stimulation of the C2 and C3 ventral rami, which carry afferents from sternocleidomastoid and trapezius, also evoked no p.s.p.s in biventer and complexus motoneurones (nineteen tested) when only the lowest threshold fibres are activated.

Vestibular nerve stimulation (Fig. 2*J*, *K*) produces i.p.s.p.s in these same motoneurones at disynaptic latencies (Wilson & Maeda, 1974). It has been suggested that

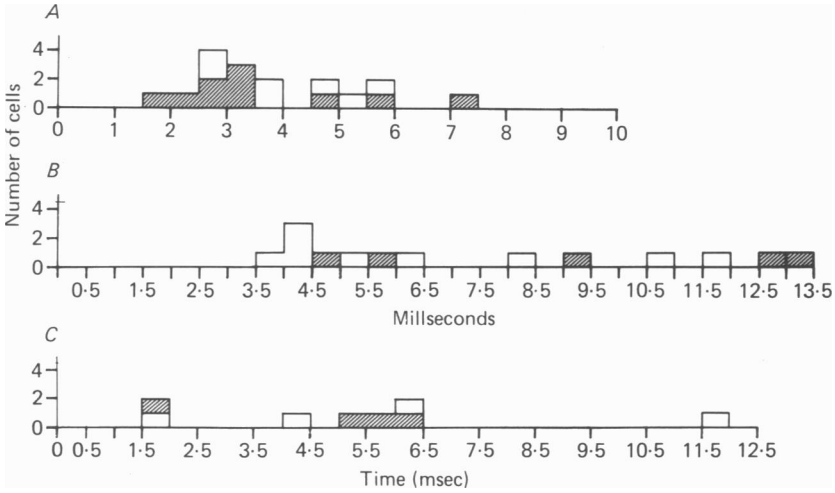

Fig. 3. *A*, central delay of i.p.s.p.s recorded in biventer and complexus motoneurones upon stimulation of C2 ventral ramus. (C2 biventer and complexus motoneurones shaded, C3 biventer and complexus motoneurones unshaded). *B*, central delay of i.p.s.p.s recorded in sternocleidomastoid motoneurones upon stimulation of the ipsilateral C2 biventer and complexus (shaded), and C3 biventer and complexus (unshaded) nerves. *C*, central delay of i.p.s.p.s recorded in trapezius motoneurones upon stimulation of ipsilateral C2 biventer and complexus (shaded) and C3 biventer and complexus (unshaded) nerves.

both the vestibulocollic i.p.s.p. and the disynaptic Ia i.p.s.p. in the lumbo-sacral motoneurones are mediated by the same transmitter (Felpel, 1972; Bradley, Easton & Eccles, 1953). Furthermore, both of these pathways have synapses located proximally on the motoneurone (Jankowska & Roberts, 1972; Rapoport, Susswein, Uchino & Wilson, 1977). Thus the presence of the vestibulocollic i.p.s.p. in this preparation makes it unlikely that the lack of an inhibitory effect upon stimulation of the lowest threshold afferents from the antagonist muscle, is due to suppression of glycinergic transmission, or to damage to the proximal portion of the motoneuronal membrane.

Increasing the stimulus strength beyond 1·4 times threshold generally gave rise to mixed synaptic potentials in these motoneurones. Fig. 2*A–D* depicts the results obtained in a sternocleidomastoid motoneurone on stimulating the C3 afferent from biventer and complexus. At 1·4 times threshold (*A*) an e.p.s.p. first becomes apparent, which at higher stimulus strength is followed by an i.p.s.p. Both of these potentials continue to increase in amplitude at stimulus strengths beyond 7 times threshold (*D*). Fig. 2*F–I* depicts a pure i.p.s.p. produced in a sternocleidomastoid motoneurone on stimulating the C2 biventer and complexus afferents. Here the i.p.s.p. does not become apparent until 2 times threshold (not shown, but see *F* and

G), and continues to increase in amplitude as the stimulus strength is increased beyond 6 times threshold (*H*, *I*). This situation also obtains in biventer and complexus motoneurones upon stimulation of sternocleidomastoid afferents (Fig. 2*E*). These results appeared consistently in all sternocleidomastoid (12/12) and biventer and complexus (19/19) motoneurones which were tested.

The central delays of the i.p.s.p.s produced in sternocleidomastoid motoneurones on stimulation of the biventer and complexus nerve were always longer than 3·5 msec (Fig. 3). In biventer and complexus motoneurones the earliest i.p.s.p. produced on stimulation of sternocleidomastoid afferents occurred at 1·6 msec, and the mean i.p.s.p., central delay was 3·8 msec (Fig. 3). When mixed effects were observed the e.p.s.p. always preceded by the i.p.s.p., and in these cases the i.p.s.p.s were reversed by injection of hyperpolarizing current, and i.p.s.p. latencies measured at the point were the i.p.s.p. and reversed-i.p.s.p. diverged.

The motoneurones of trapezius, a shoulder muscle which plays a role in extending the head (Duchenne, 1867), were also studied for reflex effects from biventer and complexus. Stimulation of biventer and complexus at strengths of up to 1·6 times threshold evokes no p.s.p.s in trapezius motoneurones (twenty-one motoneurones tested). When biventer and complexus afferents were stimulated at higher stimulus strengths e.p.s.p.s appeared more frequently (12/21) than i.p.s.p.s (8/21). The central delays of the observed i.p.s.p.s ranged from 1·6 to 7·0 msec (Fig. 3). Stimulation of C2 ventral ramus which carries muscle afferents primarily from sternocleidomastoid, but also from trapezius, produced only monosynaptic e.p.s.p.s in trapezius motoneurones.

Crossed reflex interactions between sternocleidomastoid and biventer cervicis-complexus

Stimulation of the C2 or C3 biventer and complexus nerves, near threshold for the lowest threshold fibres, evoked a sequence of hyperpolarization followed by depolarization in seventeen out of seventeen contralateral sternocleidomastoid motoneurones (Fig. 4*A*). The initial hyperpolarizing potential is increased in amplitude by passage of a depolarizing current through the recording electrode, is reversed by a hyperpolarizing current, and is thus an i.p.s.p. The depolarization, which invariably follows this i.p.s.p., is increased in amplitude by passage of a depolarizing current, and reversed by passage of a hyperpolarizing current, and is thus a disinhibition (Wilson & Burgess, 1962). These changes are shown in Fig. 4*A*, and the same sequence, an i.p.s.p. followed by a disinhibition, was seen in ten out of ten biventer and complexus motoneurones upon stimulation of the contralateral C2 ventral ramus (Fig. 4*A*), a pure afferent nerve composed primarily of fibres from sternocleidomastoid (Corbin & Harrison, 1938).

Since biventer and complexus is a mixed nerve, it is possible that the effect which stimulating it evokes in contralateral sternocleidomastoid motoneurones results from activation of sensory and/or motor fibres. However, it is improbable that this effect is mediated by motor fibres, since the same sequence of i.p.s.p.-disinhibition was evoked in biventer and complexus motoneurones by stimulation of C2 ventral ramus, a purely afferent ramus. Furthermore, stimulation of the contralateral sternocleidomastoid nerve after section of the dorsal roots produced no p.s.p.s in four out of four biventer and complexus motoneurones tested, and stimulation of the contralateral

trapezius nerve after section of the dorsal roots produced no p.s.p.s in six out of six biventer and complexus motoneurones tested. These observations suggest that it is most likely that the i.p.s.p.-disinhibition sequence results from stimulation of only sensory fibres.

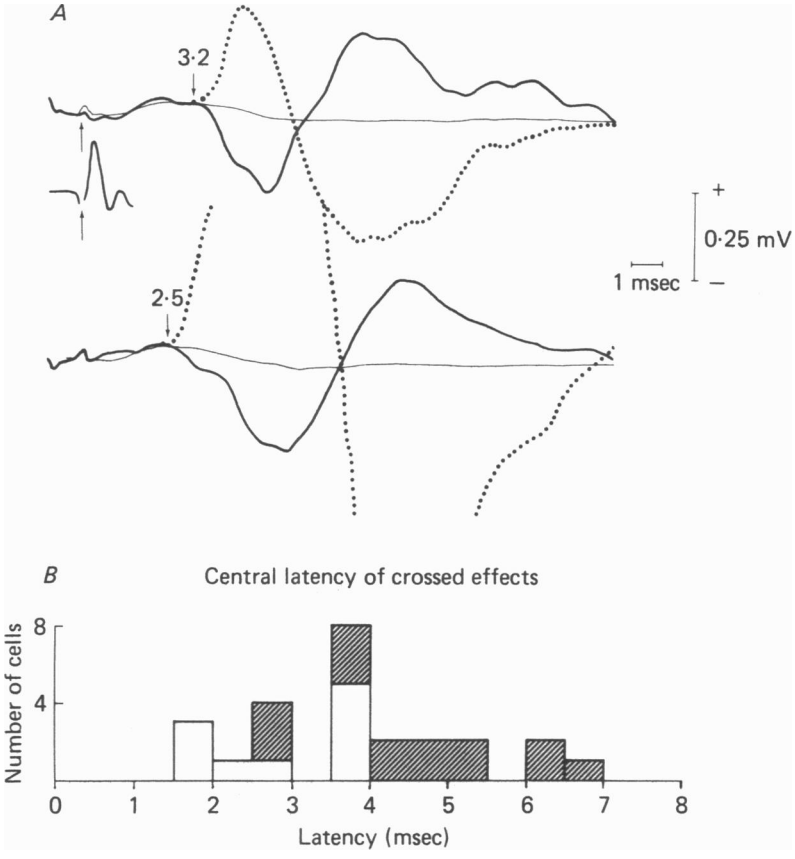

Fig. 4. *A*, effect evoked in a right C3 biventer and complexus motoneurone upon stimulation of left C2 ventral ramus. Upper tracing C2 ventral ramus stimulus at 1·1 times threshold of the lowest threshold afferents. Lower tracing at 5 times threshold. Dotted lines depict the potential recorded during injection of 35 nA of hyperpolarizing current through the recording pipette; heavy continuous line depicts the intracellular record obtained at the resting membrane potential (65 mV); thin line represents the juxtacellular fields. Inset at left depicts the afferent volley recorded at the dorsal root entry zone on the stimulated side. Upward arrows depict the stimulus artifacts. Central delay was measured from the positive peak of the afferent volley to the beginning of the i.p.s.p. (marked by downward arrow). *B*, central delay of crossed effects. Unshaded = recording in biventer and complexus motoneurones and stimulating contralateral C2 ventral ramus. Shaded = recording in sternocleidomastoid motoneurones and stimulating contralateral C3 biventer and complexus nerve.

In one experiment in which dihydro-β-erythroidine (DHE, 1 mg/kg) was injected intravenously, while recording intracellularly from a sternocleidomastoid motoneurone, the disinhibitory potential was abolished, while the i.p.s.p. which preceded it remained, and the resting potential was not altered. In this same motoneurone the monosynaptic e.p.s.p. elicited on C2 ventral ramus stimulation was in no way altered by the DHE. In addition, whereas the sequence of i.p.s.p.-disinhibition had been elicited in five sternocleidomastoid motoneurones before DHE

injection, only an i.p.s.p. was observed in four sternocleidomastoid motoneurones which were penetrated after DHE injection. The motoneurones penetrated before and following DHE injection all had similar resting potentials. The persistence of the early i.p.s.p. also suggests that the crossed i.p.s.p. is not mediated by recurrent motor-axon collaterals.

The shortest central delays of i.p.s.p.s evoked in sternocleidomastoid motoneurones by stimulation of crossed low-threshold biventer and complexus afferents, ranged

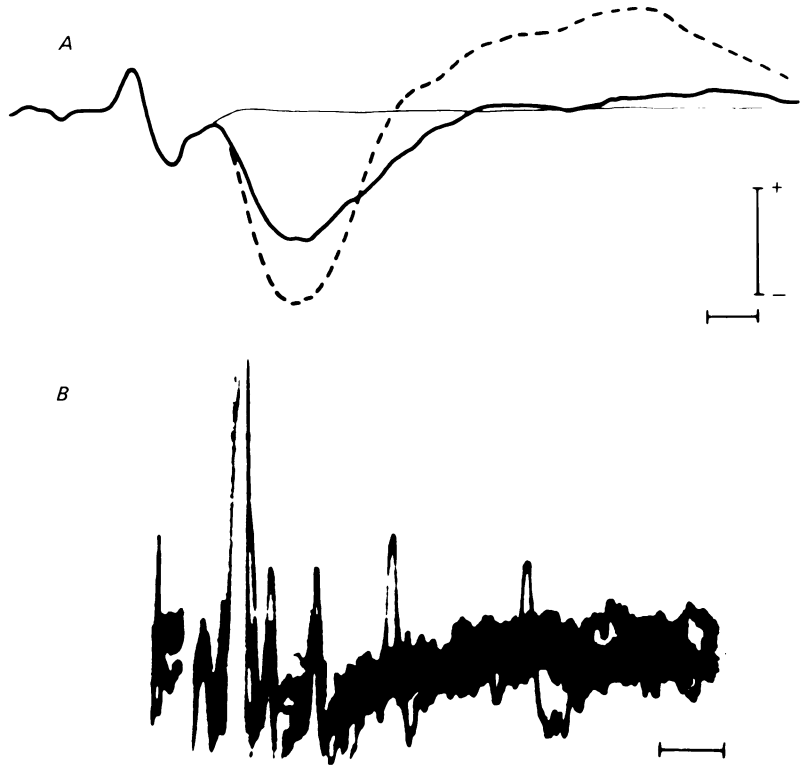

Fig. 5. *A*, intracellular record obtained in a trapezius motoneurone upon stimulation of the trapezius nerve in a deafferented preparation. The heavy continuous line depicts the record obtained at the resting membrane potential; the interrupted line depicts the record obtained upon injection of a 15 nA depolarizing current through the recording electrode; thin line depicts the juxtacellular field. Voltage calibration = 0·5 mV. *B*, extracellular record of an interneurone which fired in response to stimulation of the trapezius nerve, in a deafferented preparation. Three superimposed traces were photographed at a stimulus repetition rate of 4 Hz. Note the invariability of firing of the first two spikes of this interneurone. Stimulus artifact at 1 msec, antidromic field potential at 2 msec. Time scale: *A*, 1 msec; *B*, 2 msec.

from 2·6 to 6·6 msec, those of i.p.s.p.s evoked in biventer and complexus motoneurones by stimulation of low-threshold sternocleidomastoid afferents from 1·7 to 4·0 msec (Fig. 4*B*). On several occasions the latency of an i.p.s.p. could be shortened by stimulation with strong shocks (Fig. 4*A*), or with multiple shocks, but comparisons of latencies were always made between the shortest intervals measured (Fig. 4*B*).

Effects produced by stimulation of ventral root fibres

Three tests were employed in investigating possible effects produced in collic motoneurones by recurrent motor-axon collaterals. *A*, the deafferented muscle nerve was stimulated below threshold for the antidromic action potential, and the penetrated motoneurone studied for any p.s.p. produced. *B*, an increase in the amplitude of the afterhyperpolarization was looked for as the strength of the antidromic stimulus was increased. *C*, the deafferented muscle nerve was split into two branches, and the branch not containing the axon of the penetrated motonurone was stimulated strongly (up to 10 times the threshold of the antidromic spike).

Forty motoneurones were studied for presence of p.s.p.s. from ventral root fibres (fifteen biventer and complexus, eleven splenius, ten trapezius, four sternocleidomastoid). Of the four motoneuronal types studied, trapezius exhibited p.s.p.s most consistently, while sternocleidomastoid, biventer and complexus and splenius were remarkable for the paucity of ventral root-evoked effects which they exhibited. Ventral root-evoked p.s.p.s were not observed in any of the sternocleidomastoid motoneurones studied (0/4); in only two of the biventer and complexus (2/15) and in only three of the splenius (3/11) motoneurones studied. In contrast, most of trapezius motoneurones (8/10) exhibited ventral root-evoked i.p.s.p.s. Stimulation of a deafferented muscle nerve of a type other than that of the penetrated motoneurone failed to elicit any post-synaptic effect (0/22).

The typical ventral root-evoked effect observed consisted of an i.p.s.p. of 4–15 msec duration, occasionally followed by a depolarization (Fig. 5*A*). The late depolarization could be reversed by injection of a hyperpolarizing current, and its amplitude could be increased by injection of a depolarizing current. At times the combined i.p.s.p.-disinhibitory effect attained a duration of 25 msec. The central delays of the thirteen i.p.s.p.s observed ranged from 0·8 to 4·0 msec (measured from the positive peak of the antidromic field potential). The median i.p.s.p. latency was 1·4 msec. The i.p.s.p.s varied in amplitude from 0·2 to 1·2 mV.

In trapezius motoneurones i.p.s.p.s were observed in motoneurones with both long and short afterhyperpolarizations, and in the case in which no i.p.s.p. was observed and the afterhyperpolarization was measured, it was of short duration. In sternocleidomastoid, splenius and biventer and complexus however, i.p.s.p.s were often not observed even in cells with long afterhyperpolarizations.

Interneurones firing in response to stimulation of deafferented muscle nerves. Interneurones were found in the ventral horn, ventral to the motoneurones, which fired upon antidromic stimulation of deafferented muscle nerves. The threshold for activation of these interneurones was low, being typically equal to or lower than the threshold for the production of an antidromic action potential in a nearby motoneurone. Such interneurones were found in response to stimulation of each of the four deafferented muscle nerves stimulated.

Ventral root-activated interneurones fired their first spike at a latency as early as 0·5 msec measured from the positive peak of the antidromic field potential (Fig. 5*B*). These interneurones always fired two early spikes in response to an antidromic stimulus, even at threshold for their firing. The instantaneous frequency of firing of

the early spikes usually exceeded 1000 Hz (Fig. 5*B*). This rapid early firing was usually followed by a prolonged silent period (Fig. 5*B*), although rarely the cells continued to fire repeatedly for several milliseconds. This silent period coincided with the time of maximal disinhibition of the intracellularly recorded post-synaptic effect.

DISCUSSION

Monosynaptic e.p.s.p. from homonymous afferents

The present experiments have reconfirmed the existence of monosynaptic e.p.s.p.s from homonymous and heteronymous afferents in biventer and complexus (Wilson & Maeda, 1974; Anderson, 1977), and have extended those observations to include two other neck muscles, sternocleidomastoid and trapezius. Sternocleidomastoid, like splenius, biventer and complexus, is involved in movement of the head, and functions as a flexor and rotator; while trapezius is primarily an elevator of the scapula, and secondarily an extensor and rotator of the head.

The monosynaptic e.p.s.p. in motoneurones of the upper cervical cord results from activation of the lowest threshold afferents. The threshold of the e.p.s.p. is the same as that of the lowest threshold afferents; the e.p.s.p. increases in amplitude as more and more low-threshold afferents are recruited, and the e.p.s.p. exhibits most of its growth in amplitude at stimulus strengths below 1·6 times the threshold of the lowest threshold afferents. It is likely that the monosynaptic e.p.s.p. in cervical motoneurones, which satisfies all of the electrophysiologic characteristics of the monosynaptic Ia e.p.s.p. of lumbo-sacral motoneurones (cf. Eccles *et al.*, 1957), is likewise the result of activation of Ia fibres.

Ipsilateral interactions between biventer-complexus and sternocleidomastoid

In the present experiments, no effect could be elicited in biventer and complexus motoneurones upon stimulation of the ipsilateral, low-threshold afferents from sternocleidomastoid or vice versa. Is it possible that such an effect was overlooked?

As discussed above, the lowest threshold fibres were active in this preparation, and so were segmental interneurones. Yet, these interneuronal pathways, which were activated by stimulation of higher threshold fibres, even when facilitated did not evoke disynaptic i.p.s.p.s in the antagonist. Furthermore, since similar results were obtained despite the use of three distinct anaesthetic protocols, it is unlikely that the absence of a low-threshold i.p.s.p. between these antagonists resulted from an idiosyncratic effect of an anaesthetic.

The absence of a disynaptic i.p.s.p. from low-threshold afferents of the antagonist is due neither to selective inactivation of glycinergic pathways, nor to damage to the proximal portion of the motoneurone membrane, upon which Ia interneurones are thought to synapse. These points were demonstrated in experiments in which monosynaptic vestibulo-collic i.p.s.p.s could be elicited in biventer-complexus motoneurones, while disynaptic i.p.s.p.s could not be evoked in these motoneurones upon stimulation of ipsilateral sternocleidomastoid afferents.

Lastly, a computer averaging technique, which can easily detect a signal of 100 μV (Rapoport *et al.* 1977) was utilized in these experiments. Therefore, if a disyn-

aptic, low-threshold i.p.s.p. is present between ipsilateral sternocleidomastoid and biventer and complexus, its magnitude must be extremely small.

Disynaptic Ia i.p.s.p.s between antagonists are not a ubiquitous phenomenon in the spinal cord. Even in the hind limb reciprocal Ia inhibition appears to be restricted to motoneurones which are antagonists during flexion-extension motions of the limb, such as those performed during scratching and locomotion. For instance, disynaptic Ia inhibition is present between flexors and extensors of the hip, but it is absent between hip adductors and abductors (R. M. Eccles & Lundberg, 1958). Disynaptic Ia antagonism is absent between internal and external intercostals, which are antagonists during respiration (Sears, 1964). It is also absent between masseter and digastric, which are antagonistic jaw closers and openers (Kidokoro *et al.* 1968). As has been demonstrated in the present paper, disynaptic Ia inhibition is also absent between flexors and extensors of the head. Other than between motoneurones of the same limb which function as antagonists during flexion extension, disynaptic Ia inhibition is found only between contralateral homologous hind limb motoneurones (Perl, 1958, 1959; but see Holmqvist, 1961) and between crossed antagonists of the tail (Wilson & Lloyd, 1956; Curtis, Krnjević & Miledi, 1958). To summarize our present knowledge, it appears that the disynaptic Ia inhibitory pathway is a neural specialization which is limited to spinal circuits which mediate some functions of the limb and the tail.

Low-threshold crossed effects

Anderson (1977) demonstrated the absence of an effect in biventer and complexus and splenius upon stimulating the corresponding, homologous, lowest threshold afferents on the contralateral side. In the present experiments it has been shown that a crossed, low-threshold effect exist between sternocleidomastoid and biventer and complexus motoneurones, and vice versa. This i.p.s.p.-disinhibition sequence occurs, for the most part, at central latencies which are longer than disynaptic, yet, because of the small size of the motoneurone population studied, it is not possible to completely rule out a disynaptic path.

It may be argued that because sternocleidomastoid and biventer and complexus motoneurones are located in different spinal segments, a central latency longer than 1·5 msec is permissible for a crossed disynaptic path between them. In the lumbosacral spinal cord, whenever two motoneurones are linked by Ia reciprocal inhibition, the central latency of the i.p.s.p. is always less than 1·5 msec. This is observed even when the motoneurones are separated by several spinal segments. For example, sartorius motoneurones are located in the L4–L5 segments, while semimembranosus motoneurones are located in L6–L7 (Romanes, 1951). These two motoneurone pools are linked by Ia inhibition, and the central latencies of the i.p.s.p.s in semimembranosus upon stimulation of Ia afferents from sartorius are always less than 1·5 msec (1·3 msec, R. M. Eccles & Lundberg, 1958). The crossed Ia i.p.s.p. of tail motoneurones also occurs with a segmental delay of less than 1·5 msec (Curtis *et al.* 1958). These data from the lumbosacral cord suggest that it is not unreasonable to expect the crossed effect between biventer and complexus and sternocleidomastoid, if it were disynaptic, to occur with a central delay of 1·5 msec or less.

Effects produced by stimuluation of ventral root fibres

In cats whose dorsal roots had been cut from C1 to C7 or C8, stimulation of muscle nerves from biventer and complexus, splenius and trapezius activated inter-

neurones in the ventral horn, and occasionally evoked p.s.p.s in the respective motoneurones. The ventral root-activated interneurones were fired at central delays which were monosynaptic, measured from the positive peak of the antidromic field potential generated by nearby motoneurones, and the p.s.p.s produced in the motoneurones had central latencies which were at least disynaptic. It is thus conceivable that these interneurones are mediating the ventral root-evoked p.s.p.s in the motoneurones.

Although substantial numbers of unmyelinated sensory fibres have been described in ventral roots (Coggeshall, Clifton, Vance & Applebaum, 1975), the stimulus strengths which have been presently employed were not sufficiently strong to activate such fibres. Furthermore, the delays of the observed effects were much shorter than what would be expected of p.s.p.s produced by unmyelinated fibres. It is therefore unlikely that these antidromic effects were produced by ventral root sensory fibres, at least by fibres of the calibres which have been described in the lumbo-sacral spinal nerves. Most probably these antidromically evoked effects are mediated by motor-axon collaterals.

Aberrant, myelinated ventral root sensory fibres have also been noted to occur (see for instance Kato & Hirata, 1968; Kato & Tanji, 1971). Some of these fibres behave similarly to group II spindle afferents, while others have joint and cutaneous sensory fields. These ventral root sensory fibres are few in number, and their significance is difficult to assess. Ventral root sensory fibres in the upper cervical spinal cord were not reported by Richmond *et al.* (1976). Since it is unlikely that these investigators would have missed a prominent ventral root sensory projection, it is reasonable to assume that ventral root sensory fibres are not any more common in the upper cervical spinal nerves than they are in the lumbo-sacral ventral roots.

The latencies of i.p.s.p.s which were observed upon stimulation of deafferented muscle nerves, did not differ from the latencies with which recurrent i.p.s.p.s are observed in the lumbo-sacral spinal cord. Although the latencies of recurrent i.p.s.p.s generally observed by Eccles *et al.* (1954) varied from 1·1 to 1·8 msec, these authors state that 'probably still longer values would obtain for very small hyperpolarizations'. Indeed, the late recurrent i.p.s.p.s in the present sample were small, and would probably not have been detected without the use of a computer averaging technique.

Renshaw cells typically fire a high-frequency spike train of 30–50 msec duration (Eccles *et al.* 1954). In contrast, the ventral root-activated interneurones of the upper cervical cord fired two initial spikes, which were often followed by a silent period. Such an interneuronal firing pattern is reflected in the intracellular records from upper cervical motoneurones, as is the firing pattern of Renshaw cells in motoneurones of the lumbo-sacral cord. The ventral root generated i.p.s.p.s were usually of short duration, and were often followed by a disinhibition. If these p.s.p.s are mediated by the ventral root-activated interneurones, the disinhibition can then be attributed to the silent period of interneuronal activity.

In the lumbo-sacral spinal cord, two mechanisms have been identified which can produce disinhibition of motoneurones upon activation of recurrent collaterals. Hultborn, Jankowska, Lindstrom & Roberts (1971) have demonstrated that Renshaw cells inhibit the tonic inhibitory Ia interneurone. Clearly, activation of Renshaw cells can depolarize motoneurones via this system. The other mechanism which might be involved in Renshaw cell mediated disinhibition is Renshaw cell inhibition

of Renshaw cells (Ryall, 1970). The present results suggest that a Ia interneurone is not involved in the spinal circuit of the upper cervical segments. Therefore, the observed disinhibition must be produced by a mechanism which does not involve Ia interneurones. Thus, Renshaw cell mediated inhibition of Renshaw cells remains as one possible avenue for the production of the observed disinhibition, but other mechanisms cannot be ruled out.

The paucity of effects in these motoneurones, upon stimulating deafferented muscle nerves, was particularly striking in view of the fact that interneurones were frequently encountered which could be activated by such a stimulus, and is reminiscent of a similar finding by Sears (1964) in the thoracic spinal segments. In limb segments, recurrent i.p.s.p.s are found in all tonic motoneurones, and in half of the phasic motoneurones (Granit, Pascoe & Stag, 1957; Eccles *et al.* 1961). In the present study, recurrent effects were frequently found in the motoneurones of trapezius, a shoulder muscle whose motoneurones reside in the upper cervical spinal segments. However, in sternocleidomastoid, splenius and biventer and complexus motoneurones recurrent i.p.s.p.s were very rare, and were even absent in motoneurones with long afterhyperpolarizations. These results suggest that the recurrent pathway may not be as potent in the neck as it is in the limbs.

Recurrent inhibition is another specialization which is limited to a few spinal circuits. It appears very prominently in motoneurones which receive Ia inhibition, as is evident in the hind limbs (Eccles *et al.* 1954; Eccles *et al.* 1961) and in the forelimbs (Thomas & Wilson, 1967). It is absent from some motoneurones which do not receive Ia inhibition, such as phrenic (Gill & Kuno, 1963) and sphincter motoneurones (Mackel, 1978). Of the motoneurones examined in the present study, only trapezius, which is primarily a shoulder muscle, appears to receive a prominent recurrent effect. It is difficult to assess the role which is being performed by the occasional recurrent i.p.s.p. observed in the motoneurones of muscles involved in head movement. The present sample, because of its small size and the infrequency of recurrent effects, is not very enlightening.

The motoneurone groups which have been presently studied innervate the bulk of the collic musculature. It is clear that Ia disynaptic inhibition and recurrent inhibition are not salient reflex circuits among these motoneurones. Although several small collic muscles have yet to be studied for their reflex connexions, these muscles, because of their size, are not likely to figure prominently in effecting head movements. It would thus appear that Ia disynaptic inhibition and recurrent inhibition do not play a major role in the generation or control of head movements. The marked contrast between the present results and the many demonstrations of direct inputs from numerous descending systems onto collic motoneurones, tends to suggest that control of head movement is primarily mediated by supraspinal centres.

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