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THE MEASUREMENT OF SINGLE MOTOR-AXON RECURRENT INHIBITORY POST-SYNAPTIC POTENTIALS IN THE CAT

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SUMMARY

1. Signal averaging was used in forty experiments on low-spinal cats to measure and characterize the oligosynaptic responses of seventy-six motoneurones supplying the medial gastrocnemius muscle to the single impulses of antidromically stimulated single motor axons supplying the same muscle.

2. In thirteen experiments on chloralose–urethane anaesthetized preparations, twelve (43%) of the tested twenty-eight motoneurones exhibited a single-axon recurrent inhibitory post-synaptic potential (recurrent i.p.s.p.), as compared to sixty-four (62%) of the 103 motoneurones tested in twenty-seven animals in the absence of anaesthetic after ischaemic decapitation.

3. Single-axon recurrent i.p.s.p.s most often consisted of a single, long-lasting hyperpolarization. Ten of the recurrent i.p.s.p.s contained a second late peak of hyperpolarization. In another eight of the i.p.s.p.s, a small late depolarization was evident.

4. The distinct profiles of the recurrent i.p.s.p.s were readily distinguished from the relatively flat profiles with low noise levels in the averages of the fifty-five 'no-response' cells. The transmembrane and post-synaptic nature of the i.p.s.p.s was confirmed by extracellular control recordings taken immediately outside seven of the cells with positive responses. In addition, ten cells with positive responses were subjected to current passage during the averaging procedure. In all cases, depolarization increased and hyperpolarization reduced the amplitude of their single-axon recurrent i.p.s.p.s.

5. The mean amplitude of the responses was 12·0 μV in chloralose–urethane preparations as compared to a peak-to-peak noise level $< 6{\cdot}0$ μV in the no-response averages. Corresponding values in ischaemic–decapitate preparations were 46·2 μV and $< 7{\cdot}5$ μV, respectively.

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6. Latency, rise-time and half-width (i.e. duration at half-amplitude) values of the i.p.s.p.s were similar for chloralose–urethane and ischaemic–decapitate preparations. The average values in both preparations were 2·5, 5·6 and 19·3 ms, respectively. The latency values indicated both disynaptic and, perhaps, longer components in the recurrent i.p.s.p.s. The rise-time and half-width values were relatively similar to those reported or measured from published records for analogous composite recurrent i.p.s.p.s (i.e. responses to antidromic stimulation of the whole muscle nerve rather than single motor axons). A weak, but significant, correlation between rise-time and half-width was observed for the sixty-six single-axon recurrent i.p.s.p.s with a single negative-going peak.

7. The present results complement other recent information on selected unitary aspects of the recurrent Renshaw circuit, including Renshaw cell responses to stimulation of single motor axons (Ross, Cleveland & Haase, 1975, 1976; Van Keulen, 1981) and motoneurone responses to activation of single Renshaw cells (Van Keulen, 1981).

8. The present work demonstrates that the recurrent Renshaw circuit is particularly responsive to the discharge of single motoneurones, facilitating the study of selected features of recurrent Renshaw inhibition, such as its distribution within a single spinal motor nucleus, as described in the accompanying paper (Hamm, Sasaki, Stuart, Windhorst & Yuan, 1987).

INTRODUCTION

Since the pioneering studies of Renshaw (1941, 1946), the recurrent inhibitory circuit to motoneurones has generated considerable interest with respect to its organization and functional significance (for review: Baldissera, Hultborn & Illert, 1981). However, our understanding of this circuit is far from complete. This is due in part to the fact that most previous studies of the Renshaw pathways to homonymous, heteronymous and 'antagonistic' spinal motor nuclei have used composite activation (Burke & Rudomin, 1977) of these pathways by antidromic stimulation of whole muscle nerves. There is a need to supplement the results obtained by use of this approach with information on the unitary organization of synaptic connections within the recurrent Renshaw circuit. Such work is exemplified in the studies of Ross, Cleveland & Haase (1975, 1976) and Van Keulen (1981), which revealed a wide range in the responsiveness of single Renshaw cells to the recurrent input from single motor axons. Furthermore, by use of spike-triggered averaging, Van Keulen (1981) provided examples of the wide range of motoneuronal i.p.s.p.s which occur in response to the individual impulses of single Renshaw cells.

Averaging techniques have provided a means of analysing the monosynaptic e.p.s.p.s produced in single motoneurones by the action potentials from single spindle Ia afferent axons (Mendell & Henneman 1968; Jack, Miller, Porter & Redman, 1971). Subsequently, the spike-triggered averaging technique was shown to be appropriate for the analysis of oligosynaptic (particularly disynaptic) circuits, such as those involved in the actions of single Ia, spindle group II and tendon organ Ib axons on motoneurones (Stauffer, Watt, Taylor, Reinking & Stuart, 1976; Watt, Stauffer, Taylor, Reinking & Stuart, 1976; for review: Kirkwood & Sears, 1980). In the present

study, we have utilized signal averaging to analyse a motoneuronal response to stimulation of a single motor axon. We have termed this response a single-axon recurrent inhibitory post-synaptic potential (single-axon recurrent i.p.s.p.).

This paper describes the measurement, incidence and characterization of recurrent i.p.s.p.s produced in motoneurones supplying the cat medial gastrocnemius muscle by the impulses of single motor axons supplying the same muscle. In the accompanying paper (Hamm, Sasaki, Stuart, Windhorst & Yuan, 1987), analysis of this data reveals a topographic distribution of recurrent Renshaw inhibition within a single spinal motor nucleus.

Preliminary accounts of this work have been presented (Yuan, Hamm, Sasaki, Windhorst, Vanden Noven & Stuart, 1985; Hamm, Yuan, Sasaki & Stuart, 1987).

METHODS

Preparation

This report is based on experiments on forty adult cats weighing 2·1–4·5 kg. Most of the surgical procedures have been described in detail in previous reports from this laboratory (Watt *et al.* 1976; Botterman, Hamm, Reinking & Stuart, 1983). The only new feature was the handling of the test muscle nerve or muscle such that single motor axons could be either impaled and stimulated within the muscle nerve or selectively activated within the muscle.

Gaseous anaesthesia (halothane–nitrous-oxide–oxygen) was used for the initial surgical procedures. Two types of preparations were used. For the first, a mixture of α-chloralose (60 mg/kg) and urethane (600 mg/kg) was administered intravenously while the gaseous anaesthesia was gradually removed. This anaesthetic mixture produced a deep anaesthesia which was sustained by giving supplemental doses (at 0·1–0·2 times the original strength) as needed throughout the experiment.

In the second type of preparation, the brain was destroyed ischaemically under gaseous anaesthesia. Both carotid and both vertebral arteries were occluded, the latter pair by passing a heavy string around the vertebral column between the C1 and C2 vertebrae (technique modified from Sherrington, 1919). Brain death was confirmed at least half an hour after the arterial occlusions. It was indicated by fixed and maximally dilated pupils, the absence of reflexes (corneal, pinnal, etc.) and the general appearance of the craniofacial tissue, such as rigor mortis of the tongue musculature. If there were any signs indicating that occlusion of blood flow to the head was incomplete, a craniotomy was performed and the brain removed by suction. Following ischaemic destruction or removal of the brain, gaseous anaesthesia was discontinued.

In both preparations, the spinal cord was transected at the L1–L2 level. The L6–S2 dorsal roots were sectioned in all experiments. In most experiments (thirty-four out of forty), the animal was paralysed by the intravenous administration of gallamine triethiodine and artificially ventilated with ventilation adjusted to maintain end-expired CO_2 around 4%. Paralysis was helpful in stabilizing the spinal cord for more secure intracellular recording from motoneurones and was required when using intramuscular stimulation of motor axons (see below) to provide the stability needed for consistent stimulation of single axons.

After the surgical preparation, the animal was mounted in a rigid Goteborg-type frame. A bilateral pneumothorax was then performed. If required, metaraminol bitartrate was added to the drip to maintain blood pressure at 100–120 mmHg.

The rostral and caudal halves of the L7 ventral roots and the S1 ventral roots were gently separated and suspended over separate monopolar recording electrodes, referred to the back musculature.

The final hind-limb preparation procedure (for experiments employing intra-axonal stimulation) involved mounting the sciatic nerve on a stable platform at the mid-thigh level. This procedure facilitated the use of a stepping motor to subsequently advance a glass micro-electrode into single motor axons within the medial gastrocnemius fascicle. The sciatic nerve was pinned to a layer of wax on the platform and covered with dental-impression material (polyvinyl silicone), excluding an area for electrode penetration (Fig. 1).

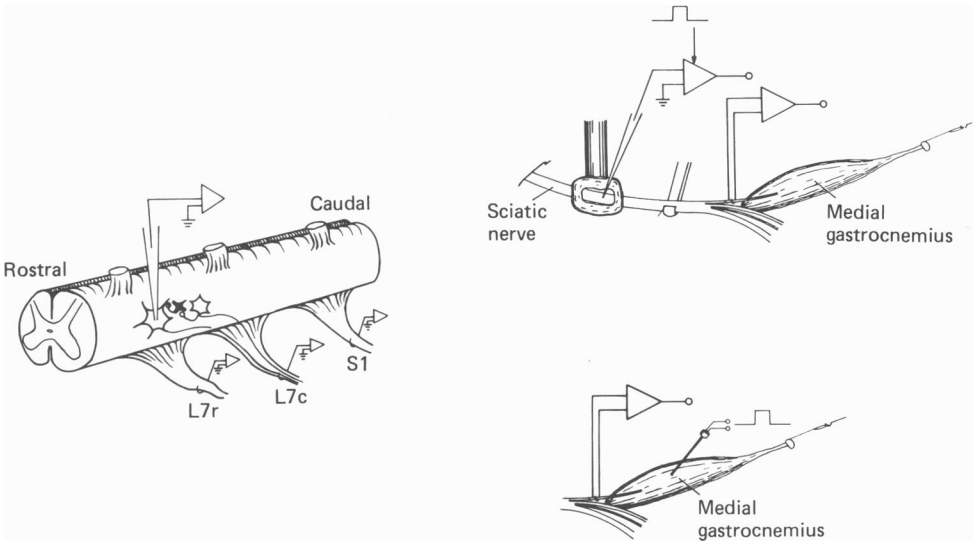

Fig. 1. Experimental arrangement for measurement of single-axon recurrent i.p.s.p.s. Left, spinal arrangements showing intracellular recording from a medial gastrocnemius motoneurone and three separate extracellular recordings from L7 rostral (r), L7 caudal (c) and S1 ventral roots. Top right, intra-axonal stimulation of single motor axons (or their myelin sheaths) innervating medial gastrocnemius, using a micro-electrode. Alternatively, single motor axons were activated intramuscularly using a fine bipolar electrode as shown at bottom right. For both techniques, the unitary nature of the stimulation was judged by observation of an extracellularly recorded action potential in one segment of the ventral roots. See text for further details.

Mineral oil pools were formed at the spinal cord and at the leg. Rectal temperature and leg-pool temperature were independently controlled at 37 ± 1 °C. With these procedures, the cord-pool temperature stabilized at 35 °C.

Stimulating and recording procedures

Axon stimulation and recording. Single motor axons innervating the medial gastrocnemius muscle were stimulated using either one of two techniques (Fig. 1). The first was by means of intra-axonal or intra-myelin penetrations using glass micro-electrodes (see Zealear & Crandall, 1982; cf. Yuan, Hamm, Reinking & Stuart, 1983). These electrodes were filled with 2 M-potassium citrate and had an impedance of 30–50 MΩ. Single axons were stimulated through the micro-electrodes using 0·1–0·5 ms pulses of anodal current. The second technique involved stimulating the motor axons intramuscularly, by use of a fine bipolar electrode (Taylor & Stephens, 1976). Satisfactory results were obtained using either commercial electrodes, or electrodes fabricated by running two leads of fine stainless-steel wire through a 25-gauge hypodermic needle. The electrode was advanced manually through the muscle tissue, stopping frequently to deliver brief (0·1 ms) stimulus pulses. At sites where motor axons were stimulated, as judged by action potentials recorded at the ventral-root level, the stimulus strength was reduced until a single spike was evident in one of the ventral-root recordings. With monopolar recordings (referred to the muscle tissue adjacent to the spinal cord) available from three ventral-root segments (Fig. 1), the action potentials of single motor axons could be observed in single traces of an oscilloscope. Thus, the intermittency and all-or-none character of the action potential could easily be noted at stimulus threshold. As stimulus strength was increased, the action-potential profile became contaminated, thereby indicating stimulation of additional axons. If no other motor axons were stimulated at a stimulus strength 2·5–3·0 times threshold for the axon, the unit was accepted for study. During the recording of single-axon

recurrent i.p.s.p.s, each of the three ventral-root recordings was checked after every 2048 sweeps to ensure that only the single motor axon was being stimulated.

In the present study, both methods of single-axon stimulation were used. In both cases, the ventral-root recordings containing the action potential of the test axon were monitored throughout the recording period. Stimulation of single axons using these two methods evoked recurrent i.p.s.p.s of similar profile and amplitude.

Motoneurone recording and stimulation. Intracellular potentials were recorded from medial gastrocnemius motoneurones using glass micro-electrodes filled with 2 M-potassium citrate. The impedance of these electrodes was 3–5 MΩ.

Medial gastrocnemius motoneurones were identified by antidromic invasion consequent to stimulation of the whole nerve to the test muscle. Following location of the medial gastrocnemius motor nucleus, the tip of the electrode was left in the immediate vicinity of a medial gastrocnemius motoneurone while a second micro-electrode was advanced into the fascicle of the medial gastrocnemius nerve at the mid-thigh level for intra-axonal stimulation, or a bipolar electrode was inserted into the medial gastrocnemius muscle for intramuscular stimulation. Once a medial gastrocnemius motor axon had been successfully impaled or a single medial gastrocnemius motor axon had been successfully stimulated intramuscularly, a motoneurone was next impaled and the axon was stimulated repetitively for a period of 5–15 min at a stimulus frequency of 6–8 Hz. The number of stimuli (sweeps) required to average a measurable i.p.s.p. ranged from 210 to 8192.

In some cases, after the average of a single-axon recurrent i.p.s.p., the input resistance and rheobase of the test motoneurone were determined by injecting current into the cell. These values were much lower than those encountered in this laboratory's previous measurements when using chloralose–urethane preparations (e.g. Hamm, Koehler, Stuart & Vanden Noven, 1985). This reduction in both sets of values was attributable to making input resistance and rheobase value measurements at the end of relatively long (5–10 min, sometimes as long as 18 min) averaging epochs and to the use of ischaemic–decapitate preparations. Consequently, no attempt was made to provisionally classify motoneurones by type (Burke, Levine, Tsairis & Zajac, 1973) according to these electrical properties (Zengel, Reid, Sypert & Munson, 1985).

In some instances, the effect of current injection was tested on single-axon recurrent i.p.s.p.s. This was accomplished by injecting depolarizing or hyperpolarizing currents (2·7–17·5 nA). A bridge circuit was used to offset the potential drop across the impedance of the micro-electrode. There was sometimes a small amount of variability in the recurrent i.p.s.p. amplitude during the time required for averaging. Thus, it was important to have a valid measurement of the control i.p.s.p. (i.e. without current injection) for comparison to that taken during current passage. This was achieved by use of a computer program to average the recurrent i.p.s.p.s at a control level of membrane potential and during depolarizing or hyperpolarizing current injection. The sequence of averaging (at 7·3 Hz) was: current plus stimulus; stimulus alone; and current alone. This sequence was then repeated in reverse order before the cycle was renewed. Application of these three conditions permitted comparison of control (stimulus alone) and test (stimulus plus current) conditions as obtained during the same period of time. The average obtained during the condition 'current alone' was subtracted from that obtained during current injection with stimulus. This procedure was employed to remove the effects of electrode polarization or other potential artifacts associated with current injection. The number of samples obtained during this procedure was kept to a minimum (< 1000 per condition) to minimize the deleterious effects of injecting an impermeable ion (citrate) during hyperpolarizing current injection (cf. Finkel & Redman, 1983).

Once the measurements on each test motoneurone were completed, another motoneurone was impaled and tested. If stable single intra-axonal or intramuscular stimulation was lost from the test axon, another axon was found and intracellular recording in the spinal cord was resumed. Proceeding in this fashion, a successful recording session typically lasted 8–12 h, and involved intracellular recording from three to ten motoneurones and intra-axonal stimulation of two to six motor axons.

Data storage and processing

On-line procedures. During each experiment, averaged wave forms were collected on-line using a 4-channel signal averager. The signals collected on the averager were: motoneurone potential (intracellular), muscle–nerve neurogram, axonal potential (intra-axonal), and the extracellular ventral-root recording containing the impulse of the test axon. Concurrently, these wave forms were

stored on FM tape in addition to the following signals: low-gain d.c. record of the test motoneurone's membrane (control) potential, trigger pulses indicating the times of stimulation of the test motor axon and injection of polarizing current into the test motoneurone, and audio record. Upon occasion, an extracellular control average was taken immediately after the intracellular motoneuronal average.

Off-line procedures. Averages of the recorded signals were subsequently made off-line, checking again to ensure that single motor axons were consistently stimulated (as judged from the ventral-root recording) and that the intracellular records were free from artifacts (such as those due to vascular pulsation). Characteristics of the single-axon recurrent i.p.s.p.s were measured from these averages. They included amplitude, latency, rise-time (from the foot to the maximum of the potential) and half-width (i.e. the interval between times at which the i.p.s.p. attained half-maximum amplitude; cf. Rall, 1967). The membrane (control) potential of the test cells was measured from the low-gain intracellular wave form. Also, the amplitude was measured of single-axon recurrent i.p.s.p.s recorded in test motoneurones at different levels of the resting potential (i.e. by current injection).

Statistical tests. The relations between selected variables described in the Results section were evaluated with standard least-square regression analyses. The Mann–Whitney U test was used to test for significant differences ($P < 0{\cdot}05$) between means of variables.

RESULTS

Identification of single-axon recurrent i.p.s.p.s

The data presented here were derived from forty successful experiments, in which 76 out of 131 medial gastrocnemius motoneurones exhibited a recurrent i.p.s.p. in response to stimulation of a single motor axon. Typical examples are shown in Fig. 2. The profiles of recurrent i.p.s.p.s were characterized by a long-lasting hyperpolarization that was clearly distinguishable from the control (pre-stimulus) portion of the average. They were also different from the profile of extracellular control averages, which were taken after intracellular averaging from seven cells which exhibited recurrent i.p.s.p.s (e.g. Fig. 2*D*, middle trace). These extracellular averages had peak-to-peak noise levels generally in the range of 3–6 μV (cf. Stauffer *et al.* 1976; Watt *et al.* 1976), thereby providing additional support for the present judgement of positive responses. Similarly, the noise levels of the intracellular averages with no i.p.s.p.s were generally $< 7{\cdot}5\ \mu$V. Thus, the profile of single-axon recurrent i.p.s.p.s was clearly distinguished from the relatively flat base line of non-response averages.

In averaging the intracellular potentials during single-axon stimulation, the averaging process was continued until the magnitude of the i.p.s.p. was judged by eye to be at least twice as great as the background noise (in the case of a response), or until the noise level was small enough so that only the smallest of i.p.s.p.s. would not have been observed (in the case of no response). For the twelve responses in the chloralose–urethane preparations, averages were based on a mean ($\pm$S.D.) of 4208 ± 2396 (range of 960–8192) sweeps. Averages were based on 1915 ± 1619 (range of 210–7000) sweeps for the sixty-four i.p.s.p.s in ischaemic–decapitate preparations. The difference in the number of sweeps reflects the smaller amplitude of the recurrent i.p.s.p.s in the chloralose–urethane preparations (see below). In comparison, the numbers of sweeps for the 'non-response' group were 3566 ± 1203 (range of 1200–5600) for the chloralose–urethane preparations and 2635 ± 855 (range of 1350–4096) for the ischaemic–decapitate preparations.

The sample sizes needed in averaging single-axon recurrent i.p.s.p.s were smaller than those used previously in averaging oligosynaptic Ia, spindle II and Ib post-synaptic potentials (p.s.p.s; Stauffer *et al.* 1976; Watt *et al.* 1976), reflecting the smaller amplitudes of the p.s.p.s in previous studies. Another indication of the

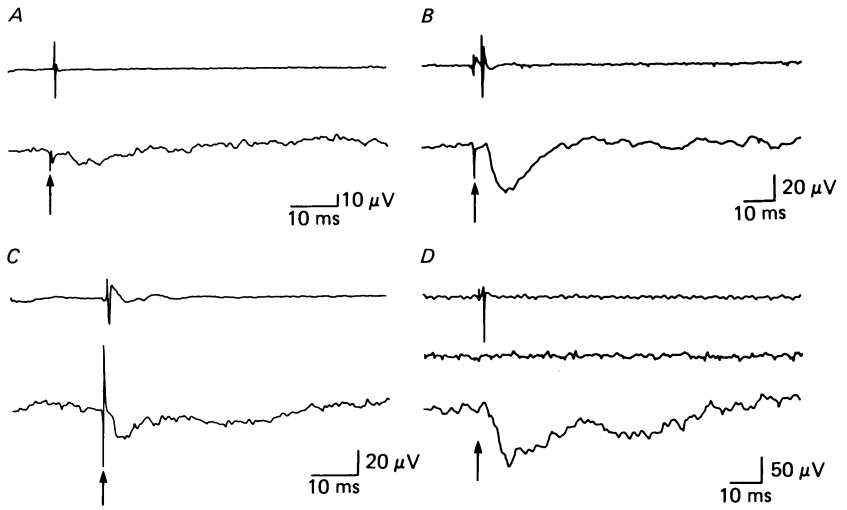

Fig. 2. Examples of single-axon recurrent i.p.s.p.s. All traces are averages triggered by activation of a single medial gastrocnemius motor axon. Upper traces are extracellular ventral-root recordings and lower traces are intracellular motoneurone recordings. Arrows indicate stimulus onset. Averages based on 1024 (*A*), 4096 (*B*), 4096 (*C*) and 1900 (*D*) stimuli (sweeps). Middle trace in *D* is an extracellular recording, taken from just outside the test motoneurone. This control average suggests that the recurrent i.p.s.p. was a true transmembrane hyperpolarization. *A* and *C*, records from chloralose–urethane preparations. *B* and *D*, responses from ischaemic–decapitate animals. Two negative-going peaks are observable in the profile of the *C* and *D* recurrent i.p.s.p.s, as seen in ten of the seventy-six recurrent i.p.s.p. averages. Note different voltage calibrations and time scales.

relatively secure transmission through the Renshaw interneuronal pathway in comparison to other spinal circuits is based on our observation that the distinctness of the recurrent i.p.s.p.s tended to increase progressively during the averaging period with only a small degree of variability, like disynaptic Ia i.p.s.p.s but unlike Ib i.p.s.p.s, in which considerable variability in the form of the response could occur during averaging (Watt *et al.* 1976). This implied constancy of interneuronal transmission and recurrent i.p.s.p. amplitude is supported by observations on a sample of the ten largest recurrent i.p.s.p.s in our study. The variation in amplitude of these potentials in successive quarters of the averaging period was no more than could be attributed to background synaptic noise (Yuan, 1986; Hamm, Yuan, Sasaki, Windhorst & Stuart, 1986).

Effects of current passage

Establishment of the post-synaptic nature of the recurrent i.p.s.p.s requires demonstration of the sensitivity of these potentials to changes in membrane potential. In the present study, this demonstration was accomplished by using a variable stimulus regimen and current injection through the micro-electrodes (see Methods). Fig. 3 shows typical changes in recurrent i.p.s.p. amplitude produced by injecting depolarizing and hyperpolarizing currents into the motoneurone. The amplitude of the i.p.s.p. enlarged as the membrane (control) potential was moved

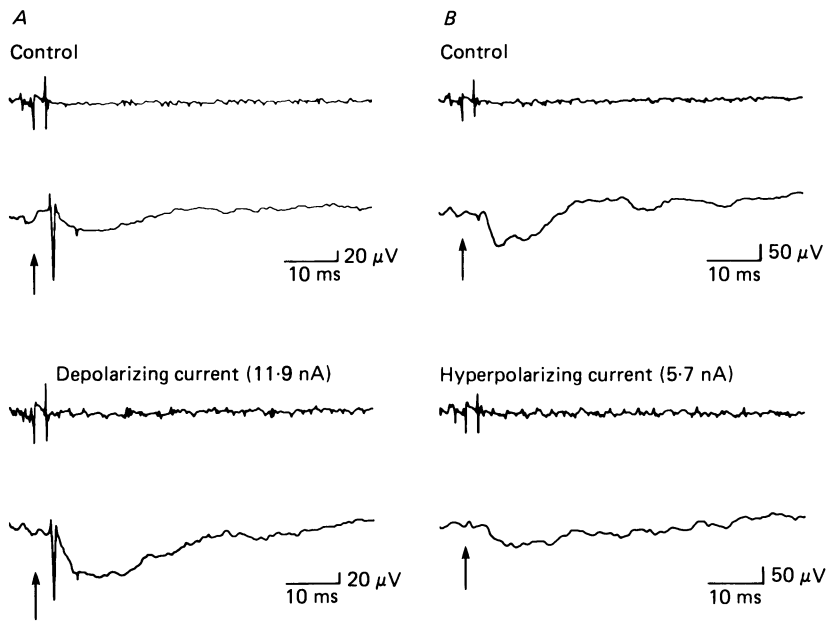

Fig. 3. Effects of current passage on single-axon recurrent i.p.s.p.s. Traces and arrows are as in Fig. 2. *A* and *B* are from different experiments. *A*, upper traces are control averages of 350 sweeps. Lower traces are corresponding averages during depolarizing current passage (11·9 nA). *B*, upper traces are control averages of 256 sweeps. Corresponding lower traces are during hyperpolarizing current injection (5·7 nA). Amplitude changes during current injection are indicative of post-synaptic responses.

away (depolarized) from the equilibrium potential for the ions bearing 'inhibitory current' (i.e. Cl^- and possibly K^+; cf. Eccles, 1964; Jankowska & Roberts, 1972). In contrast, i.p.s.p. amplitude decreased when the membrane potential was hyperpolarized to approach this equilibrium potential. These results were obtained consistently for eighteen different levels of current injection in ten cells, indicating that single-axon recurrent i.p.s.p.s are largely attributable to post-synaptic effects with little contribution from such potential effects as dysfacilitation.

We did not obtain any i.p.s.p. reversals when injecting current. The magnitude of current injection was limited to avoid its deleterious effects over long averaging intervals. Moreover, the current requirements for recurrent i.p.s.p. reversal are increased by the relatively distal locations of synapses on motoneurones from Renshaw cells, as recently demonstrated in studies in which Renshaw cells and motoneurones were stained with horseradish peroxidase (Fyffe, 1986), confirming results of previous electrophysiological studies (Burke, Fedina & Lundberg, 1971).

Components of the recurrent i.p.s.p.

Cullheim & Kellerth (1981) have demonstrated two negative-going components in the profile of composite recurrent i.p.s.p.s, one of short duration which they presumed to be mediated by glycine, and one long-duration component presumably mediated by γ-aminobutyric acid (GABA). In the present study, a feature of ten of the seventy-six single-axon recurrent i.p.s.p.s was the presence of two negative-going peaks in the potentials (e.g. Fig. 2*C* and *D*) separated by intervals ranging from

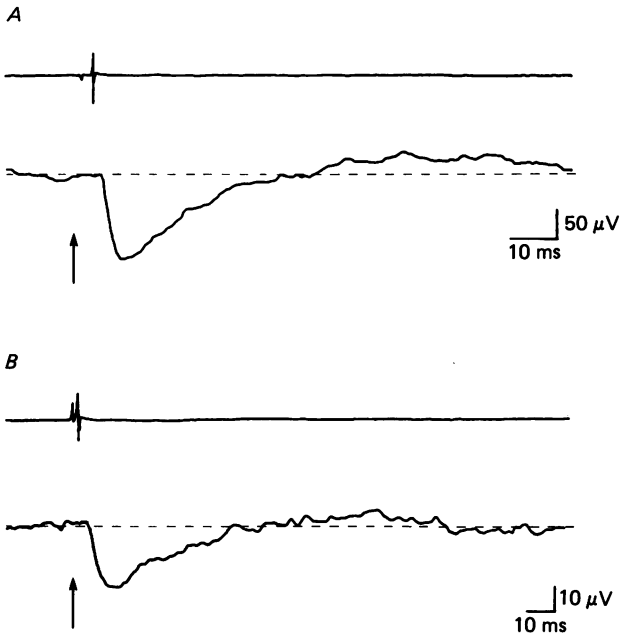

Fig. 4. Examples of depolarizations after single-axon recurrent i.p.s.p.s. Traces and arrows as in Figs. 2 and 3. *A* and *B* are from different experiments with both sets of averages based on 2048 sweeps. Horizontal dashed lines indicate membrane (control) potential level to emphasize the depolarizations that were observed after both recurrent i.p.s.p.s. Note different voltage calibrations and time scales.

12·0 to 38·4 ms. The time courses of these potentials are similar to those in Cullheim & Kellerth's (1981) observations on composite recurrent i.p.s.p.s. However, alternative mechanisms, such as repetitive Renshaw-cell spikes, could conceivably contribute to such potentials.

Another feature in the present work is shown in Fig. 4. In eight of the recurrent i.p.s.p.s, a depolarization was clearly observed after the hyperpolarization. Future studies will be required to determine if this late depolarization is attributable to dysinhibition, or to other mechanisms which are as yet unidentified. Possible sources of dysinhibition have been previously demonstrated in the inhibition of Ia reciprocal inhibitory interneurones by Renshaw cells (e.g. Fig. 8 in Hultborn, Jankowska & Lindström, 1971), and in the mutual inhibition between Renshaw cells (Ryall, 1970, 1981).

These late components were recorded both in cats that were paralysed and those that were not. Consequently, the possibility can be excluded that these components are an artifact resulting from the motor unit contraction which follows axonal stimulation in the non-paralysed preparation.

Characteristics of recurrent i.p.s.p.s

Amplitude. As indicated previously, our results show that chloralose–urethane has a depressant effect on the recurrent Renshaw circuit. This effect is clearly shown

TABLE 1. Characteristics of single-axon recurrent i.p.s.p.s

Preparation (membrane potential)	Amplitude (μV)	Latency (ms)	Rise-time (ms)	Half-width (ms)
Chloralose–urethane (58 ± 7·6 mV)	12·0 ± 6·9 (3·5–27·4) n = 12	3·5 ± 1·9 (1·0–6·7) n = 10	6·5 ± 3·2 (2·1–9·9) n = 9	24·1 ± 12·4 (8·6–43·7) n = 11
Ishchaemic–decapitate: 1 (57 ± 7·5 mV)	38·5 ± 17·3 (17·0–74·0) n = 18	2·1 ± 0·7 (1·0–3·2) n = 18	6·4 ± 2·7 (2·3–11·4) n = 16	18·7 ± 8·6 (10·0–33·5) n = 18
Ischaemic–decapitate: 2 (56 ± 8·3 mV)	49·3 ± 51·5 (10·2–332·3) n = 46	2·4 ± 1·0 (0·7–5·5) n = 43	5·3 ± 2·5 (2·5–11·9) n = 46	18·3 ± 10·6 (7·5–51·1) n = 45
Ischaemic–decapitate: 1 + 2 (56 ± 8·1 mV)	46·2 ± 44·7 (10·2–332·3) n = 64	2·3 ± 1·0 (0·7–5·5) n = 61	5·5 ± 2·6 (2·3–11·9) n = 62	18·5 ± 1·0 (7·5–51·1) n = 63
Both preparations (57 ± 7·9 mV)		2·5 ± 1·2 (0·7–6·7) n = 71	5·6 ± 2·7 (2·1–11·9) n = 71	19·3 ± 10·8 (7·5–51·1) n = 74

Membrane potential and recurrent i.p.s.p. values expressed as mean ± S.D. (with range of recurrent i.p.s.p. values in parentheses). n = number of motoneurones tested. Latency was measured as the interval from the isoelectric point in extracellular ventral-root recording of axon spike to the foot of the i.p.s.p. Rise-time was taken as time from the foot to the peak of the i.p.s.p. while half-width was the interval between the two half-amplitude points. Intra-axonal stimulation was used to activate recurrent i.p.s.p.s in chloralose–urethane and one (1) group of ischaemic–decapitate preparations. Intramuscular stimulation was used in a second (2) set of experiments from the latter preparations.

in Table 1 and Fig. 5. This depression produced by chloralose–urethane was evident also in the incidence of recurrent i.p.s.p.s. In chlorase–urethane preparations, twelve i.p.s.p.s were found in recordings from twenty-eight motoneurones (43 %); the incidence in ischaemic–decapitate preparations was sixty-four of 103 (62 %). In one experiment, a single-axon recurrent i.p.s.p. with an amplitude of 67 μV was recorded in an ischaemic–decaptitate cat. 5 min after the intravenous injection of a small dose of chloralose (10 mg/kg)–urethane (60 mg/kg), the amplitude of the averaged i.p.s.p. reduced to 28 μV, with no deterioration in the recording conditions. Further such studies would be of interest, using graded doses of chloralose, urethane and their combination.

Table 1 includes separate groupings of values for the single-axon recurrent i.p.s.p.s obtained in response to intra-axonal and intramuscular stimulation (the former coming from chloralose–urethane and ischaemic–decapitate preparations and the latter exclusively from ischaemic–decaptitate ones). There was no significant difference between the amplitudes of i.p.s.p.s recorded in response to these two forms of stimulation, a finding which serves to validate the technique used to ensure the unitary nature of intramuscular stimulation (see Methods). The larger mean value (49·3 *vs.* 38·5 μV) for the intramuscular-stimulation group of i.p.s.p.s was attributable in large part to the sample containing two atypically large values of 192 and 322 μV (plotted as > 100 μV in Fig. 5). The significance of these values was more likely to have been that they were recorded from cells whose spinal locations were quite close

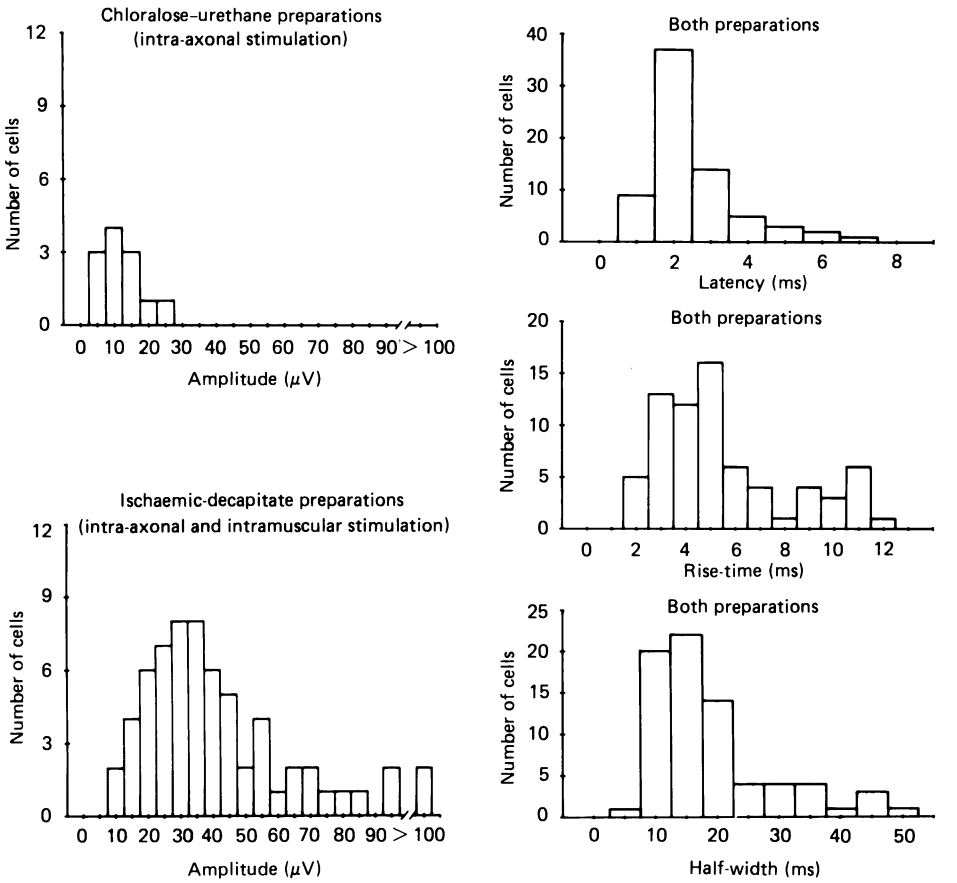

Fig. 5. Distributions of characteristics of single-axon recurrent i.p.s.p.s. Left, histograms of the amplitude of recurrent i.p.s.p.s in the two types of preparation, as activated by intra-axonal or intramuscular stimulation. Right, values for recurrent i.p.s.p. latency, rise-time and half-width, with values of chloralose–urethane and ischaemic–decapitate animals combined in individual histograms, since their individual values were similar (cf. Table 1).

(< 200 μm) to the cell whose axon was being stimulated (see Fig. 2 in accompanying paper, Hamm *et al.* 1987), rather than being a response to a particular technique of single-axon stimulation.

Presentation of i.p.s.p. amplitudes requires consideration of the motoneurones' membrane potentials, not only due to the direct dependence of i.p.s.p. amplitude on membrane potential as demonstrated above, but also due to the influence of motoneurone condition on p.s.p. amplitude (Jack *et al.* 1971; for discussion of this issue, see also Watt *et al.* 1976). Variability due to this factor was limited by restricting recordings in most cases to motoneurones having resting potentials of 50 mV or more. The mean values of the membrane (control) potential for 'response' and 'no response' groups from chloralose–urethane preparations were 58 and 57 mV,

respectively. Analogous values from ischaemic–decapitate preparations were 56 and 55 mV, respectively. Thus, single-axon recordings were made at comparable membrane potentials, on average, for the various conditions and are readily comparable. However, no significant relation could be detected in the present sample between membrane (control) potential and recurrent i.p.s.p. amplitude ($r = 0{\cdot}21$; $P > 0{\cdot}05$), probably due to the large amount of variance introduced by other determinants of amplitude.

Latency. On the basis of existing (albeit indirect) evidence (Watt *et al.* 1976; Baldissera *et al.* 1981), a monosynaptic recurrent effect on the same or on an adjacent motoneurone would be expected to have a latency of < 0·7 ms from impulse arrival at the ventral-root entry zone to onset of the post-synaptic motoneuronal response. Polysynaptically mediated responses would have latencies > 0·7–1·0 ms. However, sharp distinctions between di- and tri-synaptic latencies are not possible on the basis of latency ranges in the 0·7–2·5 ms range (Watt *et al.* 1976; Luscher, Ruenzel, Fetz & Henneman, 1979). Table 1 shows that the mean latencies of the two groups of recurrent i.p.s.p. values for ischaemic–decapitate preparations (i.e. as produced by intra-axonal and intramuscular stimulation) were 2·1 and 2·4 ms respectively, thereby indicating an oligosynaptic pathway.

For the chloralose–urethane preparations, the mean latency was longer at 3·5 ms, but it is possible that the smaller signal-to-noise ratios of these recurrent i.p.s.p. averages made the latency measurements less reliable than those from ischaemic–decapitate preparations. Consequently, even disynaptic pathways may display latencies appreciably longer than 2·5 ms in some cases. However, there was no significant association between i.p.s.p. amplitude and latency for values obtained from *both* chloralose–urethane ($r = -0{\cdot}24$, $P > 0{\cdot}05$) and ischaemic–decapitate ($r = -0{\cdot}19$; $P > 0{\cdot}05$) preparations.

The measurement of latency was an important issue in the current study, because monosynaptic connections between motor-axon recurrent collaterals and motoneurones have been demonstrated in a morphological study employing horseradish peroxidase staining (Cullheim, Kellerth & Conradi, 1977). Moreover, a recent study reported that stimulating thin filaments of the phrenic nerve following section of dorsal roots produced excitatory effects in phrenic motoneurones at short, fixed latencies (Khatib, Hilaire & Monteau, 1986). Such effects were diminished by application of nicotinic blocking agents, indicating that monosynaptic recurrent connections were responsible for this excitation. To date, we have not observed an electrophysiological counterpart to such a 'direct' (monosynaptic) connection which would presumably be excitatory, as judged by the depolarizing effects of acetylcholine on motoneurones (Zieglgansberger & Reiter, 1974) and implied by the results of Khatib *et al.* (1986). In our data, the shortest latency was 0·7 ms, which is not too long for a monosynaptic connection but also not too short for a disynaptic one.

Rise-time. The wide range of rise-times for the total sample of single-axon recurrent i.p.s.p.s (2·1–11·9 ms) is similar to published values of rise-time for composite recurrent i.p.s.p.s in barbiturate-anaesthetized preparations, which typically range from 3 to 10 ms (e.g. Eccles, Fatt & Koketsu, 1954). As with other composite p.s.p.s (Watt *et al.* 1976), these values would be dominated by the faster components which,

as Table 1 shows for the present data, can rise as quickly as 2·1 ms. In the present sample of rise-times, there were no significant differences between values for chloralose–urethane preparations and the two groups (intra-axonal and intramuscular stimulation) from ischaemic–decapitate preparations ($P > 0{\cdot}05$). Within each of these groups and for the combined values, there were no significant associations ($P > 0{\cdot}05$) between rise-time and amplitude.

For the previous sample of oligosynaptic single-sensory-axon p.s.p.s (Stauffer *et al.* 1976; Watt *et al.* 1976), the rise-time values were quite similar when using impulses from different afferent species as the trigger spikes. These values were, on average, about 30 % of the present value of $5{\cdot}6 \pm 2{\cdot}7$ ms. This comparison suggests that the interneuronal connections and behaviour encountered in the spike-triggered averaging of single-motor-axon recurrent i.p.s.p.s, or the characteristics of i.p.s.p.s produced by single interneurones in the Renshaw pathway, are substantially different to those involved in the mediation of single-sensory-axon p.s.p.s.

Half-width. Eccles *et al.* (1954) attributed the long duration of recurrent i.p.s.p.s evoked by antidromic volleys to the high-frequency repetitive discharge of Renshaw cells. While some Renshaw cells display prolonged bursts in response to activation of collaterals of single motor axons (Ross *et al.* 1975, 1976; Van Keulen, 1981) such bursts would not be expected in the majority of Renshaw cells. None the less, as shown in Table 1, the half-widths of single-axon recurrent i.p.s.p.s were similar in duration to those of recurrent i.p.s.p.s produced by composite activation.

The half-width values were similar in chloralose–urethane and ischaemic–decapitate preparations. Their grouped mean value of 19·3 ms was probably associated with a full duration of about 29 ms, in that spot measurements of the full recurrent i.p.s.p. duration (made on thirty-five cells, all with i.p.s.p. amplitudes > 12·8 mV) revealed the half-width to be approximately 67 % of the full duration. A table of representative half-width or full-duration values has not been published for composite recurrent i.p.s.p.s. When such values are mentioned, they usually range from about 20 to 40 ms (e.g. as taken from Burke & Rudomin, 1977), thereby suggesting a half-width of about 13–27 ms. Thus, even conceding the uncertainties of these comparisons, it would seem that single-axon recurrent i.p.s.p.s have, on average, a similar duration to that of composite recurrent i.p.s.p.s.

A feature of monosynaptic Ia e.p.s.p. profiles is the accuracy with which their half-widths can be predicted from their rise-times, as based on several factors related to the electrotonic characteristics of the motoneurone and the time course of the synaptic current (for review: Rall, 1967; Burke & Rudomin, 1977). Given that the time course of oligosynaptic p.s.p.s is determined by the pattern of interneuronal discharge in addition to the above-mentioned factors, this relation between rise-time and half-width in such p.s.p.s appeared less likely (cf. Munson, Fleshman & Sypert, 1980), and it has not been tested for in previous studies. However, in the present study, for the sample of sixty-six single-axon recurrent i.p.s.p.s with a single negative-going peak, a weak correlation between rise-time and half-width ($r = 0{\cdot}25$) was shown to be significant ($P < 0{\cdot}05$). It remains to be determined how the sign and strength of this correlation result from characteristics of the discharge of Renshaw cells and the electrotonic characteristics of motoneurones.

DISCUSSION

The present results demonstrate that recurrent i.p.s.p.s produced by activity of a single motor axon can be recorded readily using averaging techniques, indicating the effectiveness with which Renshaw cells can transmit the discharge of individual motoneurones to other motoneurones of the same pool. The characterization of these single-axon recurrent i.p.s.p.s requires some general discussion of the role of interposed neurones in the recording of single-axon p.s.p.s. In addition, the information obtained in this study, in conjunction with that from previous studies on cell-to-cell interactions in the recurrent Renshaw circuit, permits an analysis of the properties of the elements of this circuit and their interactions.

The spike-triggered averaging of oligosynaptic p.s.p.s

Since the 1968 report of Mendell & Henneman, averaging techniques have been used widely to study monosynaptic e.p.s.p.s, post-synaptic population potentials (p.s.p.p.s) and field potentials in a variety of neuronal systems within the C.N.S (for review and selected subsequent *ad seriatim* developments, see: Fetz, Henneman, Mendell, Stein & Stuart, 1979; Kirkwood & Sears, 1980; Brown, Tapper & Craig, 1981; Berger & Averill, 1983; Brink, Harrison, Jankowska, McCrea & Skoog, 1983; Lucas, Cope & Binder, 1984). However, the use of averaging to analyse oligosynaptic circuits within the C.N.S. has been limited to: (1) demonstration of presumed reciprocal Ia i.p.s.p.s and Ib and spindle group II e.p.s.p.s and i.p.s.p.s (Stauffer *et al.* 1976; Watt *et al.* 1976); (2) fragmentary (albeit valuable) information on Ib p.s.p.p.s (Luscher *et al.* 1979); and (3) identification, in behaving monkeys, of corticomotoneuronal cells whose impulses affect forelimb muscle activity, as revealed by averaging intramuscularly recorded e.m.g. activity (Fetz, Cheney & German, 1976; Kasser & Cheney, 1985). The clear-cut results in these latter studies suggest the importance of activity in interposed neurones. In this case, the trigger spike, acting through monosynaptic (and probably disynaptic) connections with motoneurones, produces intermittent increases or decreases in their activity which lead in turn to an obligatory change in activity of muscle cells. Thus, in this particular pathway, voluntarily activated motoneurones and pre-motoneurones are the neuronal network that 'intervenes' between the trigger spike and the averaged muscle-cell activity. The particularly effective use of averaging in revealing oligosynaptic responses in this pathway implicates the importance of the activity level of the interposed neurones, which is set in this case by the C.N.S. at a level appropriate for the desired motor behaviour.

At the present time, it is not technically feasible to rigorously control interneuronal excitability at levels appropriate for the measurement of single-motor-axon recurrent i.p.s.p.s and single-sensory-axon p.s.p.s. However, in the present study, the simple conversion from use of chloralose–urethane to ischaemic–decapitate preparations had a dramatic effect in enhancing the efficacy of averaging for study of recurrent oligosynaptic connections. It can be anticipated that this enhancement would be heightened in preparations with intact dorsal roots and by other procedures that augment interneuronal excitability, like the use of preparations with lesions that release selected reflex pathways (e.g. Rymer, Houk & Crago, 1979) and use of

pharmacological agents like L-DOPA (L-β-3,4-dihydroxyphenylalanine; Grillner, 1981) and 4-aminopyridine (Jankowska, Lundberg, Rudomin & Sykova, 1977) to prime selected circuits. In these ways, the value of averaging can be enhanced for the study of a variety of oligosynaptic circuits within the C.N.S. and peripheral ganglia.

The force of the above comments would not be increased by detailed consideration of why the present study revealed a depressant effect of chloralose–urethane on the recurrent Renshaw circuit. The over-all literature on the central actions of chloralose involves 'a multitude of conflicting reports' (Winters & Spooner, 1966). This statement is certainly true for the recurrent Renshaw circuit, which Hultborn and collaborators found resistant to chloralose depression (e.g. Hultborn, Jankowska, Lindström & Roberts, 1971; Fedina & Hultborn, 1972; Fedina, Hultborn & Illert, 1975) in contrast to the results of Haase & Van Der Meulen (1961) and Biscoe & Krnjevic (1963). The two latter studies differ concerning the site of the depressant effect, one focusing on the excitatory recurrent collateral-Renshaw-cell synapse (Haase & Van der Meulen, 1961) and the other on the inhibitory Renshaw-cell-motoneurone connection (for review: Yuan, 1986). The literature on the central actions of urethane is not so much controversial as sporadic and fragmentary, and apparently non-existent regarding its mixture with chloralose. In view of these various uncertainties, it is sufficient simply to emphasize that the present work showed that the ischaemic–decapitate preparation is far more appropriate than the chloralose–urethane one for the measurement of single-axon recurrent i.p.s.p.s.

Interactions between individual neurones in the recurrent Renshaw circuit

To date, this study and three preceding ones (Ross *et al.* 1975, 1976; Van Keulen, 1981), together with some unpublished data of L. Van Keulen cited by Baldissera *et al.* (1981; see their Fig. 2*C*), have provided information on the response of single Renshaw cells or motoneurones to the central orthodromic stimulation of single motoneurones or Renshaw cells or to the peripheral antidromic stimulation of single motor axons. Features of the various analyses are summarized in Table 2. In the discussion that follows, these results are compared in order to analyse the behaviour of this interneuronal circuit and assess the extent to which its behaviour can be decomposed according to its individual components.

Motoneurone to Renshaw cell. In considering the transmission of action potentials from motor axon collaterals through Renshaw cells, several types of information are needed. These include: (1) the amplitude and time course of e.p.s.p.s produced in Renshaw cells by the activation of single motor axons; (2) the relation between such e.p.s.p.s and the consequent increase in the probability of discharge of the affected Renshaw cells: and (3) the number of Renshaw cells which receive synapses from collaterals of a motor axon and, in turn, synapse on the motoneurone under investigation.

Direct information on the first of these points is not available, although Walmsley & Tracey (1981) have recorded e.p.s.p.s in a few Renshaw cells using graded stimulation of ventral root filaments. These recordings revealed e.p.s.p.s of 1 mV or less in amplitude having durations in excess of 50 ms in some instances. Such a prolonged e.p.s.p. in itself could account for the prolonged time courses of single-axon

TABLE 2. Unitary analysis of the recurrent Renshaw circuit

Test	Response number and characteristics				
	No. of cells	No. of spikes			Reference
Motoneurone to Renshaw cell	3	1–15			1
	11	1–3			2
	7	< 1–13			3, 4
		Amplitude (μV)	Rise-time (ms)	Half-width (ms)	
Renshaw cell to motoneurone	12	2–54	2–12	7–14	4
Motor axon to motoneurone	12	4–27	2–10	9–44	5 (chloralose–urethane)
	64	10–332	2–12	8–51	5 (ischaemic–decapitate)

Response characteristics are presented as the ranges of reported (or measurable) values. References: 1 and 2, Ross *et al.* 1974, 1976, respectively (using unanaesthetized decerebrate preparations with a lumbosacral deafferentation); 3, L. Van Keulen, unpublished measurements (presented in Baldissera *et al.* 1981; their Fig. 2*C*); 4, Van Keulen (1981), measurements on pentobarbitone–urethane preparations that were presumably intact and 5, present work on chloralose–urethane and ischaemic–decapitate preparations (with a low spinal transection and a lumbosacral deafferentation). No. of cells = number of Renshaw-cell responses or motoneurone averages. No. of spikes = number of Renshaw cell responses to a single motoneuronal impulse. Van Keulen's (1981) motoneurone sample apparently included nine responses to a 'double' Renshaw-cell discharge (i.e. see his Figs. 1*B* and 2*C*).

recurrent i.p.s.p.s, provided that such prolonged e.p.s.p.s are also evoked by the action of single motor-axon collaterals, as seems likely, and that Renshaw cells discharge throughout most of the time course of this e.p.s.p.

Previous studies of the relationship between the profile of an e.p.s.p. and the resulting peaks of the cross-correlogram (indicating the increase in probability of discharge) have provided evidence that the cross-correlogram peaks may have a form similar to that of the potential itself (Moore, Segundo, Perkel & Levitan, 1970), the derivative of the potential (Knox, 1974; Knox & Poppele, 1977; Fetz & Gustafsson, 1983), or a combination of both (Kirkwood & Sears, 1978, 1982; Gustafsson & McCrea, 1984). Consideration of the varied results of these studies prompts the conclusion that the relation between e.p.s.p. profile and probability of discharge depends upon the amplitude of the signal in relation to the background synaptic noise (cf. Kirkwood, 1979; Bishop, Reyes & Fetz, 1986). Considering the expected small amplitude of motor-axon-collateral effects on Renshaw cells, the apparent low firing threshold of these cells (< 1 mV), and their brief after-hyperpolarizations (Eccles, Eccles, Iggo & Lundberg, 1961; Jankowska & Lindström, 1971; Hultborn & Pierrot-Deseilligny, 1979), one would expect that the probability of discharge of Renshaw cells in response to input from single motor-axon collaterals would be elevated for a period of time comparable to that of the duration of the e.p.s.p. Such expectations have been met by the limited results from studies concerning actions of single motoneurones on single Renshaw cells (Table 2). These

studies show that single Renshaw cells exhibit a wide range of responsiveness to the recurrent impulses of single motor axons; from a subtle change in excitability level (Van Keulen, 1981; his Fig. 2*D*) to a burst of thirteen spikes in response to each motoneurone impulse during motoneurone activation at 3 Hz (Baldissera *et al.* 1981; their Fig. 2*C*).

Information on the number of Renshaw cells interposed between one motoneurone and another motoneurone is not available at this time. Additional information which is needed for a full understanding of the recurrent Renshaw pathway, but is missing at this stage, includes measurement of: (1) the range of single-motor-axon monosynaptic e.p.s.p. amplitudes, which now appears feasible, following the work of Walmsley & Tracey (1981); and (2) the responses of Renshaw cells at varying levels of excitation to the recurrent impulses of a single motoneurone, as also now appears possible (*vide supra*). Even without this information, existing knowledge suggests that the antidromic impulses of a single motor axon can have a powerful excitatory effect on one or more Renshaw cells, such that single-axon recurrent i.p.s.p.s can be readily recorded in motoneurones.

Renshaw cell to motoneurone. Van Keulen's (1981) measurements of twelve motoneurones' single Renshaw cell i.p.s.p.s included an amplitude range of 2–54 μV with a mean of 12·7 μV. Our measurements of rise-time and half-width from his published illustrations yield ranges of 2–12 ms and 7–14 ms, respectively. These values for amplitude are less and for duration are greater than those for the Ia inhibitory interneurone i.p.s.p.s from Jankowska & Roberts (1972). The differences are probably attributable in part to the strong possibility that these interneurones project onto the soma of motoneurones, whereas Renshaw cells project more distally onto the dendritic tree (Smith, Wuerker & Frank, 1967; Burke *et al.* 1971; Fyffe, 1986). Other mechanisms might also come into play, of course, like a longer duration of conductance change or the possibility that there are two types of Renshaw cells producing post-synaptic inhibition in motoneurones, one using glycine to mediate a rapid i.p.s.p. and one using GABA to mediate a much longer one (Cullheim & Kellerth, 1981). (However, unpublished results obtained by L. Van Keulen in Kernell's laboratory indicated that single Renshaw cells can produce i.p.s.p.s of both short and long duration in separate motoneurones; D. Kernell, personal communication.) The much smaller amplitude values for Van Keulen's (1981) single-Renshaw-cell i.p.s.p.s as compared to Jankowska & Roberts' (1972) single-Ia-inhibitory-interneurone i.p.s.p.s may reflect a different mechanism but, more probably, are largely attributable to the small sample sizes in the two studies and the use of different anaesthetic regimens (pentobarbitone–urethane by Van Keulen *vs.* chloralose by Jankowska & Roberts). Certainly, the size of the largest single-Ia-inhibitory-interneurone i.p.s.p. measured by Jankowska & Roberts (220 μV) seems possible for a single-Renshaw-cell i.p.s.p., if the present results are taken into account, as argued below.

Motor axon to motoneurone. For oligosynaptic circuits within the C.N.S., an averaged p.s.p. is a reflection of the magnitude and timing of the change in the probability of firing of the interposed neurones, as caused by the trigger spike, and of the p.s.p.s produced by these interneurones in the test cell (cf. Watt *et al.* 1976; their Fig. 13). Considering information given in the studies which have been cited above, the profiles of single-axon recurrent i.p.s.p.s in the present study are comparable, at least

qualitatively, with those expected from the i.p.s.p.s produced in motoneurones by the discharge of single Renshaw cells and the probability of Renshaw-cell discharge in response to motoneurone discharge. Further insight into the influence of one motoneurone's discharge on another is provided by comparing the amplitude of the present sample of i.p.s.p.s from chloralose–urethane preparations and the analogous monosynaptic i.p.s.p.s produced in motoneurones by activation of single Renshaw cells (Table 2). The ratio of values for these two p.s.p.s is also of interest when compared to the corresponding ratios, again from roughly comparable preparations, of oligosynaptic single-Ia-axon i.p.s.p.s (Watt *et al.* 1976; their Table 2) and monosynaptic single-Ia-inhibitory-interneurone i.p.s.p.s (*vide supra*, data of Jankowska & Roberts, 1972).

For the recurrent pathway, the mean ratio of amplitude values is 0·94 for motor-axon recurrent i.p.s.p. and Renshaw-cell i.p.s.p., as based on values of 12·0 μV and 12·7 μV, respectively. For the reciprocal Ia pathway, the corresponding ratio is 0·09, as based on a single-Ia-axon i.p.s.p. mean of 4·9 μV and single-Ia-inhibitory-interneurone mean of 57·4 μV. The 10·4-fold larger value of the recurrent circuit's ratio (i.e. 0·94/0·09) implies that the Renshaw-cell pool is 10 times as likely to respond to the impulses of the recurrent collaterals of a single motor axon than the reciprocal Ia-inhibitory pool is to respond to the impulses of the collaterals of a single Ia axon. Moreover, given the strong response of at least some Renshaw cells to activation of single motor-axon collaterals, the provisional conclusion can be drawn that relatively few Renshaw cells are interposed between motoneurone pairs, a position which is consistent with the restricted topographic distribution of single-axon recurrent i.p.s.p.s demonstrated in the accompanying paper (Hamm *et al.* 1987).

In summary, the current data on unitary aspects of the recurrent Renshaw circuit, while based on small sample sizes and use of animal preparations under vexatiously different forms of anaesthesia and different extents of surgical reduction, none the less suggest that the circuit is particularly responsive to the discharge of individual motoneurones.

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