J. Physiol. (1979), **294**, *pp.* 105–122
With 8 text-figures
Printed in Great Britain

SEGMENTAL AND DESCENDING CONTROL OF THE EXTERNAL URETHRAL AND ANAL SPHINCTERS IN THE CAT

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(*Received 18 October 1978*)

SUMMARY

1. The present work concerns the contribution of the somatic central nervous system to two viscero-somatic reflexes, micturition and defecation. Descending and segmental actions and properties of the motoneurones innervating the striated external urethral and external anal sphincters were studied with intracellular recording in male cats, under chloralose anaesthesia.

2. Motoneurones innervating the external urethral and external anal sphincters were intermingled and most strongly concentrated in the lateral part of the ventral horn in the S2 segment of the spinal cord.

3. Stimulation of the S1 to S3 ipsilateral dorsal roots or of the homonymous pudendal nerve branches showed that less than half of the sphincter motoneurones receive monosynaptic excitatory connexions from low threshold afferents.

4. The after-hyperpolarization recorded in the external urethral and external anal sphincter motoneurones was relatively short lasting, not long lasting as would have been expected for motoneurones innervating slow-twitch, tonic type muscles.

5. There was no evidence for recurrent inhibition in pudendal motoneurones innervating the external urethral and external anal sphincters.

6. Descending excitation and inhibition to the sphincter motoneurones originated in the nucleus reticularis gigantocellularis of the medullary reticular formation. The descending reticulospinal actions are comparable to those observed in hind limb motoneurones.

7. It is suggested that the segmental reflex connexions play a role in controlling bladder and rectal continence. The descending actions studied also modulate the segmental reflex actions and may provide voluntary control of the sphincter muscles.

INTRODUCTION

The bladder and rectum and their sphincters receive their innervation from the autonomic nervous system and from the somatic central nervous system. Both parasympathetic and sympathetic divisions of the autonomic nervous system innervate the smooth musculature of the bladder and rectum (Langley & Anderson, 1895,

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0022-3751/79/4850-0784 $01.50

1896). The central nervous system innervates the striated musculature of the urethra and anal canal through the deep branches of the pudendal nerve (Evans, 1936), an ordinary mixed somatic nerve carrying large and small myelinated fibres (Griffiths, 1895).

The present experiments were designed to study some connexions to, and properties of somatic sphincter motoneurones. For example, intrafusal muscle spindles, similar to those present in other striated muscles, are present in the external anal sphincter (Walker, 1959; Chennels, Floyd & Gould, 1960) and in the external urethral sphincter (Garry & Garven, 1957; Todd, 1964). Increases in e.m.g. activity and contraction of the sphincter muscles and associated striated perineal muscles can be observed when the sphincters are distended (Evans, 1936; Langworthy, Kolb & Lewis, 1940; Bishop, Garry, Roberts & Todd, 1956; Garry, Roberts & Todd, 1959). The question arises whether there is a monosynaptic contribution to these stretch evoked responses, as is the case for the hind limb muscles (Lloyd, 1943; Eccles, Eccles & Lundberg, 1957*a*, *b*). It will be shown that monosynaptic connexions to external urethral and external anal sphincter motoneurones do indeed exist.

The sphincter muscles are striated, red, tonic, slow-twitch type muscle fibres (Evans, 1936; Langworthy *et al.* 1940; Bishop *et al.* 1956; Garry & Garven, 1957). In the hind limb segments motoneurones innervating slow-twitch muscles have prominent recurrent inhibition (Eccles, Fatt & Koketsu, 1954; Granit, Pascoe & Steg, 1957; Eccles, Eccles, Iggo & Ito, 1961) and long lasting spike after-hyperpolarization (Eccles, Eccles & Lundberg, 1958; Kuno, 1959). The results will show that, surprisingly, this is not the case for sphincter motoneurones.

Studies of the descending control of the bladder or rectum have mainly dealt with the smooth musculature of the bladder or rectum (Kuru, 1965). A purely 'sphincter' brain stem area has only been described for the external urethral sphincter: stimulation of the ventrolateral ponto-medullary reticular formation led to an increased e.m.g. activity in the external urethral sphincter (Kuru, Koyama & Ozaki, 1963; Koyama, Ozaki & Kuru, 1966). The present experiments will show that both the external urethral and external anal sphincters receive descending control from localized supraspinal (reticular) areas. The supraspinal descending connexions to external urethral and external anal sphincter motoneurones are comparable to those received by hind limb motoneurones.

METHODS

Experiments were performed on thirty-three adult male cats, anaesthetized first with an I.M. injection of Vetalar (ketamine hydrochloride, Parke-Davis, 22 mg/kg), followed immediately by an I.V. injection of α-chloralose (50 mg/kg). The experimental animals were paralysed with gallamine triethiodide (Flaxedil, Davis & Geck) and respired artificially. A cannula was inserted into one of the carotid arteries and mean arterial pressure was monitored by means of a mercury manometer. When necessary, a solution of 5% dextrose in saline 0·9% or a solution of metaraminol bitartate 40 μg/ml. (Aramine, Merck, Sharp & Dome) was administered I.V. to maintain an arterial pressure of at least 90 mmHg. Rectal temperature was monitored and maintained between 37 and 39 °C by an infra-red lamp.

The m. gluteus maximus was removed and the pudendal nerve exposed in the ischio-rectal cavity. The deep branches of the pudendal nerve, which innervate the external urethral and external anal sphincters, were dissected free and prepared for antidromic and orthodromic

stimulation. For recording, a dorsal laminectomy was performed between the spinal vertebrae L5 and S1. For stimulating, a small dorsal laminectomy was performed between L1 and L2 in order to permit placement of stimulating electrodes close to the reticulospinal tracts in the ventromedial and ventrolateral funiculi of the spinal cord. In addition an occipital craniotomy was performed and the cerebellum removed by aspiration, exposing the brain stem to permit visual placement of a longitudinal 12-membered array of concentric, bipolar stimulating electrodes.

Recording and stimulating procedures. Rectangular constant current pulses (0·1 msec, 2–5/sec) were applied to the brain stem, spinal cord and peripheral nerves. Bipolar platinum hook electrodes were used for dorsal root stimulation and for antidromic and orthodromic stimulation of the peripheral pudendal nerve branches. An array of three electrolytically sharpened, lacquer-insulated tungsten wires (about 300 μm in diameter) with exposed tips of up to 300 μm was used to stimulate the upper lumbar spinal cord. The stimuli (100–200 μA) were applied monopolarly; an alligator clip in the back muscles was used as a common indifferent electrode. To stimulate the brain stem a 12-membered, 3×4 stimulating array of bipolar concentric electrodes (Rhodes Medical Instruments, SNE 100) was lowered into the brain stem under visual control to allow stimulation of the midline, medial ponto-medullary reticular formation and neighbouring brain stem areas. Typically the array was positioned so that the four medial electrodes stimulated mid-line structures while the other electrodes (four on each side), located 1·5 mm lateral to the medial ones stimulated ipsilateral and contralateral brain stem areas. The three most anterior electrodes usually were in the nucleus reticularis pontis caudalis and the most posterior electrodes in the caudal part of the nucleus reticularis gigantocellularis. Brain stem stimuli consisted of 0·1 msec, 100–200 μA pulses applied between the tip of a bipolar electrode and the concentric annular electrodes located 0·75 mm dorsal to the tip. The stimulator was arranged so that either the tip or annulus could be connected to its cathodal output. This permitted the activation of neural elements in the vicinity of either the tip or the annulus.

For intracellular recording, glass micropipettes filled with 2 M-K acetate saturated with fast green FCF were used. Pipettes with an impedance of 3–5 MΩ were selected for recording. Conventional circuits were used for recording and passing current through the micropipette. The time constants for recording were routinely 5 msec. They were increased to 500 msec for recording of spike after-hyperpolarizations.

Motoneurones innervating the external urethral and external anal sphincters were antidromically identified. The following criteria for antidromicity were used: sharp all-or-none threshold, constant latency, ability to follow high frequency, repetitive stimulation and absence of synaptic activity before the spike. Once the neurones were identified, they were located by iontophoresing dye extracellularly from the tip of the dye-filled electrodes, according to the method of Thomas & Wilson (1965). Following their identification the neurones were penetrated. Only motoneurones with stable resting potentials of at least 40–50 mV were accepted. After amplification a DC signal from the micropipette was displayed on the oscilloscope and plotted on an inkwriter (Brush Recorder Mark II) as a monitor of neuronal membrane potential and an AC signal was displayed on the oscilloscope and led to a PDP/45 computer for signal averaging. When a motoneurone with a stable resting potential was impaled, the computer was used to average its response to 25–50 stimuli applied to each of the brain stem electrodes in turn, to the spinal electrodes and to the peripheral nerves or dorsal roots.

At the end of the experiments electrolytic lesions were made to localize the brain stem and spinal cord stimulating electrodes, by passing a 20–25 μA cathodal current for 30 sec through each electrode. The animal was perfused with 10% formol saline, the brain stem and spinal cord removed, and after further fixation, 100 μm frozen sections were cut in the plane of the stimulating electrodes. The locations of the lesions were subsequently determined in thionin stained sections. The terminology of Brodal (1957) was used in the localization of the brain stem stimulating electrodes relative to the boundaries of the various reticular nuclei.

RESULTS

Localization of the motoneurones innervating the external urethral and anal sphincters

Identified sphincter motoneurones were localized by ejecting dye from the micropipette near the cell body to make a mark like that illustrated in Fig. 1*A*, *B*. Fig. 1*C* illustrates the location of several dye marks in the S2 segment of the spinal cord. As indicated by these marks, the motoneurones innervating the external urethral and anal sphincters are located in the lateral area of the sacral ventral horn which corresponds to Rexed's (1954) lamina IX.

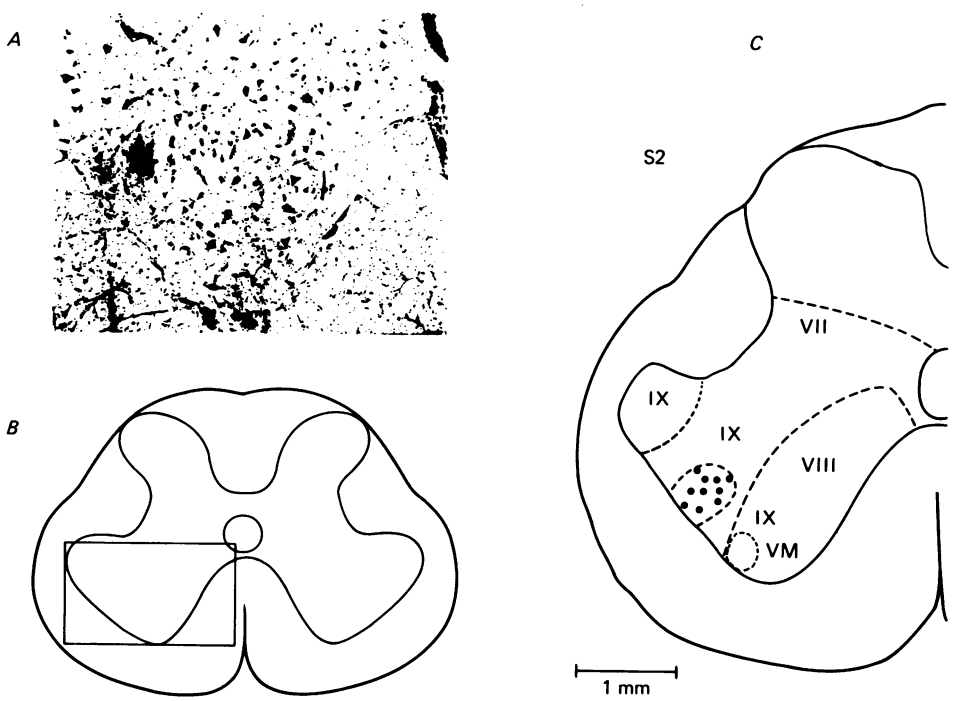


Fig. 1. Location of the motoneurones innervating the external urethral and external anal sphincters. *A*, photomicrograph of a fast green dye spot in the ventrolateral horn of the S2 spinal cord. *B*, section through the S2 segment of the spinal cord from which the photomicrograph in *A* was taken. Rectangle indicates the area shown in *A*. *C*, reconstruction of the area where several dye spots were located. This was done by superimposing several sections containing a dye spot. Each dot corresponds to a dye spot marking the location of a sphincter motoneurone.

Segmental reflex connexions

In five experiments the ipsilateral dorsal roots were cut from S1 to S3 and mounted for stimulation. Fig. 2 *B* and *C* gives examples of intracellular recording of monosynaptically evoked e.p.s.p.s following ipsilateral dorsal root stimulation. A response occurring at 1 msec or less after stimulus onset was considered monosynaptic. Using this criterion, monosynaptic responses could be evoked in fourteen out of thirty-two cells, as can be seen from the latency histogram in Fig. 3*A*. Several other motoneurones responded slightly later, perhaps also monosynaptically. The majority

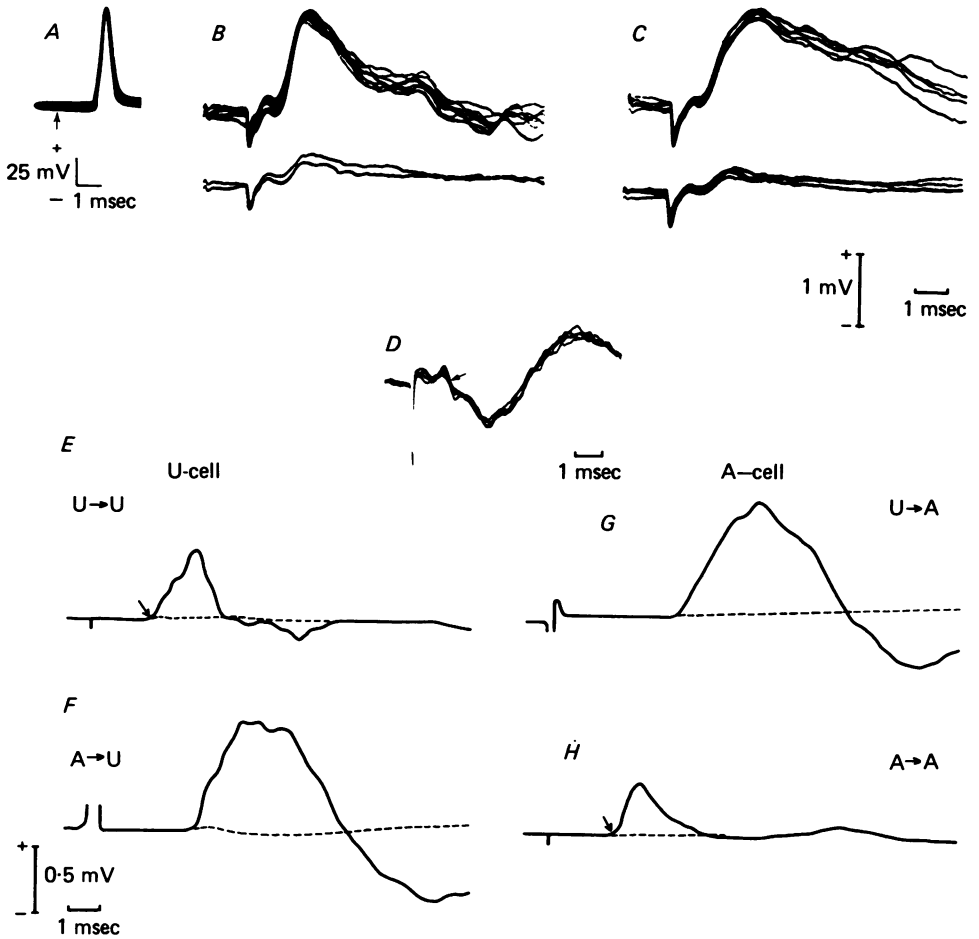


Fig. 2. Intracellularly recorded responses following stimulation of ipsilateral dorsal roots and peripheral nerve branches. *A*, the antidromic spike for the cell in *B*. Arrow indicates stimulus onset. Calibration underneath. *B* and *C*, the oscillographic recordings in two sphincter motoneurones following ipsilateral S2 dorsal root stimulation. The lower traces in *B* and *C* show the extracellular fields. Calibration for *B* and *C* underneath *C*. *E–H*, computer averaged responses obtained in two sphincter motoneurones following stimulation of pudendal nerve branches. *E* and *F*, a cell innervating the external urethral sphincter (U-cell). In *E* the response when the peripheral nerve branch containing the motor axon was stimulated (U → U). In *F* the response recorded in the same cell, but when the peripheral nerve branch innervating the external anal sphincter was stimulated (A → U). In *G* and *H*, recordings from a motoneurone innervating the external anal sphincter (A-cell). In *G* the response when the nerve to the external urethral sphincter was stimulated (U → A). In *H* the response when the nerve branch containing the motor axon was stimulated (A → A). *D* is an oscillographic recording showing the arriving triphasic volley, recorded at the dorsal root entry zone, following stimulation of the nerve branch to the external urethral sphincter. The arrow indicates the positive-negative transition part used to determine the time of arrival of the volley at the cord dorsum (1·4 msec in this case). The arrows in the averages indicate the beginning of the monosynaptic e.p.s.p.s. The dotted lines are the averaged extracellular fields. Calibration under *F* the same for all averaged records.

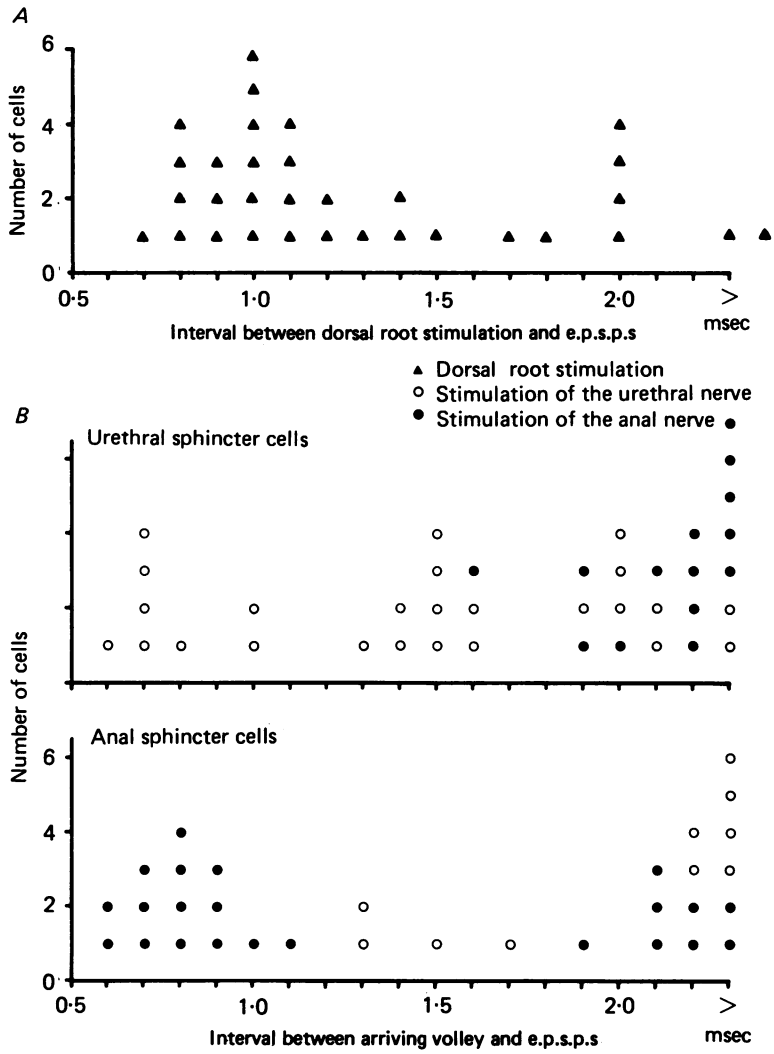


Fig. 3. Latencies and central delays of the responses obtained following stimulation of the ipsilateral dorsal roots and following stimulation of the peripheral nerve branches. *A*, the latencies of intracellularly recorded e.p.s.p.s following stimulation of the ipsilateral dorsal roots (▲). *B*, the central latencies of intracellularly recorded e.p.s.p.s in identified external urethral and external anal sphincter motoneurones. The symbols refer to the values obtained following stimulation of the nerve innervating the external urethral sphincter (○) and following stimulation of the nerve innervating the external anal sphincter (●). > indicates e.p.s.p.s appearing at latencies longer than 2·3 msec.

of the monosynaptic responses were obtained following stimulation of the ipsilateral S2 dorsal roots. Sometimes they were obtained following ipsilateral S1 dorsal root stimulation.

The origin of the monosynaptic connexions to sphincter motoneurones was studied in eleven experiments in which branches of the pudendal nerve were stimulated. Recordings were obtained from twenty-five cells innervating the external urethral

sphincter and from twenty-two cells innervating the external anal sphincter. After antidromic identification the peripheral nerve branch containing the motor axon was stimulated below motor axon threshold, to see if any low threshold afferent connexions were present. One difficulty with this procedure is that the threshold for evoking an orthodromic response was often close to the threshold for the activation of the motor axon. Hyperpolarizing current was therefore usually injected into the cell to prevent the antidromic spike from invading the soma.

Fig. 2*E–H* gives examples of the responses obtained following stimulation of pudendal nerve branches. *E* and *F* show responses recorded in an external urethral sphincter motoneurone (U-cell), once following stimulation of the nerve branch containing the motor axon (U → U) and once following stimulation of the nerve branch to the external anal sphincter (A → U). Similarly *G* and *H* show responses of a motoneurone innervating the external anal sphincter (A-cell), following stimulation of the anal (A → A) and urethral (U → A) branches. The central delays from the arrival of the afferent volley at the cord (Fig. 2*D*) to the onset of the response in *E* and *H* were 0·6–0·7 msec, i.e. these responses were monosynaptic.

Fig. 3*B* summarizes the central latencies of responses obtained in a number of external urethral and external anal motoneurones. Eight of twenty-five cells innervating the external urethral sphincter and thirteen of twenty-two cells innervating the external anal sphincter showed monosynaptic responses upon stimulation of the nerve branch containing the motor axon. Motoneurones which did not show monosynaptic responses showed later, polysynaptic excitatory responses. Stimulation of the other nerve branch (not containing the motor axon) gave only polysynaptic, most frequently excitatory responses. Occasionally there were inhibitory responses, or no responses at all.

The size of the monosynaptic responses was small, usually not exceeding 0·5 mV. (Small monosynaptic e.p.s.p.s, of the order of 100–200 μV, could be brought out with the computer averaging technique.) The size of the polysynaptic e.p.s.p.s was bigger, often more than 1·0 mV.

Duration of the spike after-hyperpolarization

The duration of the spike after-hyperpolariztion was measured in fifteen cells innervating the external urethral sphincter and in twelve cells innervating the external anal sphincter. No differences were found between the two types of motoneurones. Fig. 4 gives an example of spike after-hyperpolarization and a summary of the values obtained. The duration of the spike after-hyperpolarization was measured from the point where the falling phase of the spike crossed the baseline towards negativity to the point where the negativity came back to the baseline (see arrows in Fig. 4). These durations ranged from 50 to 100 msec, with a peak between 70 and 80 msec. They were thus in the range of after-hyperpolarizations observed for motoneurones supplying fast-twitch muscles in the hind limbs (Eccles *et al.* 1958; Kuno, 1959).

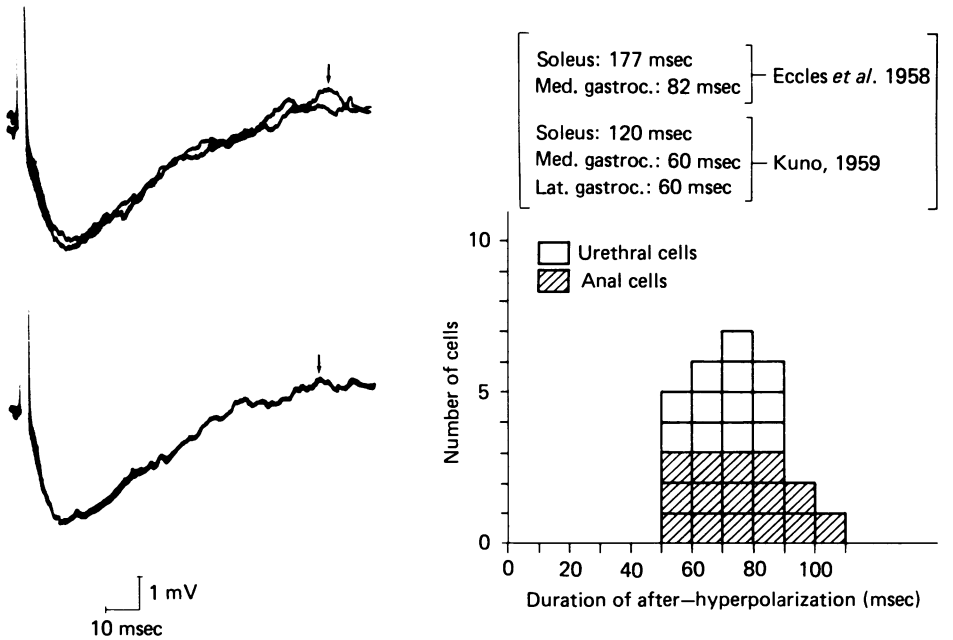


Fig. 4. Duration of the spike after-hyperpolarization in motoneurones innervating the external urethral and external anal sphincters. On the left side, two examples of spike after-hyperpolarizations. The arrows indicate where the negativity reaches the base line again. On the right side, a summarizing histogram of the spike after-hyperpolarizations for twenty-seven sphincter motoneurones. The inset above shows the average values obtained in hind limb motoneurones.

Recurrent inhibition in external urethral and external anal sphincter motoneurones

In three experiments, nine motoneurones (five external anal sphincter cells, four external urethral sphincter cells) were tested for recurrent effects. The ipsilateral L7 to S4 dorsal roots were cut. The nerve containing the motor axon, the nerve branch not containing the motor axon or the whole pudendal nerve were stimulated with single shock, multiple shocks and high frequency stimulation, below threshold for antidromic activation. In order to enhance any inhibitory effects, the motoneurones were depolarized by injecting depolarizing current into the cell body. No evidence was found for any recurrent inhibitory effects in the motoneurones innervating the external urethral or external anal sphincters.

Descending effects obtained upon brain stem stimulation

To determine which descending pathways might establish monosynaptic connexions with sphincter motoneurones, a series of experiments were performed in which the spinal cord was divided into four quadrants, which were mounted on bipolar hook electrodes for stimulation. Fast, monosynaptic e.p.s.p.s were obtained upon stimulation of the ipsilateral ventrolateral quadrant whereas stimulation of the ipsilateral dorsolateral quadrant or contralateral ventrolateral quadrants gave only longer latency, apparently polysynaptic responses. Similarly, massive stimulation

of the ipsilateral dorsolateral quadrant failed to produce any short latency descending responses. These observations indicated that the corticospinal or rubrospinal tracts are not responsible for the short latency monosynaptic descending excitation of sphincter motoneurones.

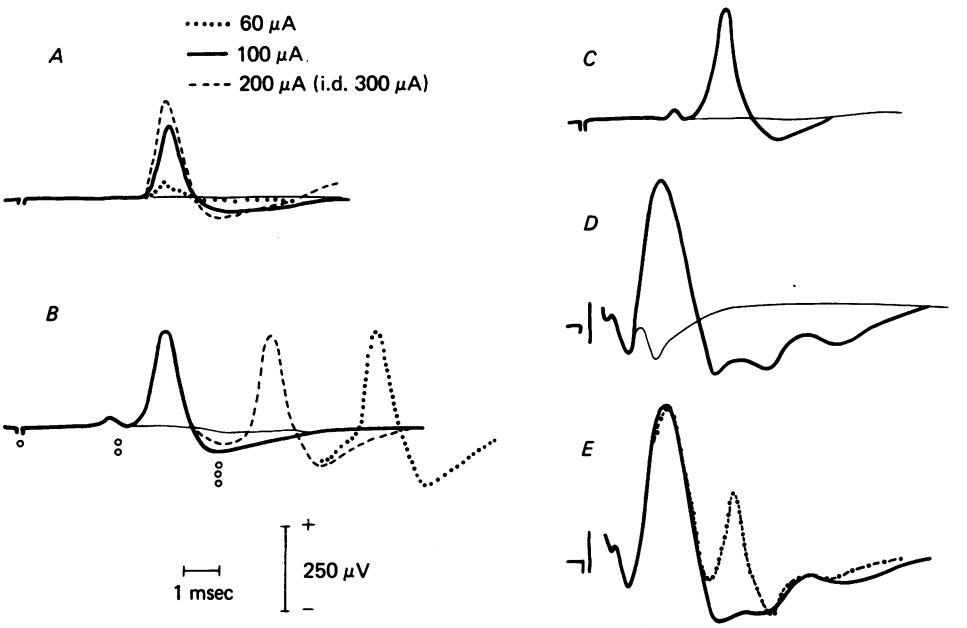


Fig. 5. Computer averaged responses recorded in an external urethral sphincter motoneurone following brain stem stimulation. *A*, gradation of the response upon increasing stimulus strength. *B*, responses recorded following single, double and triple shocks (indicated by the open circles under the record). *C*, *D*, *E*, collision test. *C*, response to brain stem stimulation. *D*, response following stimulation at high lumbar level. *E*, collision when brain stem and lumbar stimuli are delivered at the same time. The interrupted line in *E* represents the sum of *C* and *D*. The thin lines in *A*, *B*, *C*, *D* are the extracellular fields. Resting potential of the motoneurone: 60–70 mV.

Responses of external urethral and external anal sphincter motoneurones to brain stem stimulation were studied in twelve experiments. Direct excitatory responses were obtained upon stimulation of the mid-line and ipsilateral pontomedullary brain stem. An example of a monosynaptic e.p.s.p. recorded in an external urethral sphincter motoneurone is shown in Fig. 5. Because of its fast falling phase, this e.p.s.p. appears to be followed by an i.p.s.p. Increasing the stimulus strength did not cause any latency shifts (Fig. 5*A*) and Fig. 5*B* shows that during multiple shock stimulation the responses sum with no change in size or latency, an indication of monosynaptic transmission. A collision test showed that the descending response from the brain stem (Fig. 5*C*) could be collided with the response from a high lumbar electrode (Fig. 5*D*) when both stimuli occurred at the same time (Fig. 5*E*). The interrupted line in Fig. 5*E* shows the summation of *C* and *D* which would be expected if collision did not occur. Similarly, when both volleys were timed to arrive simultaneously at the recording site, no summation of the responses could

be observed (not illustrated). Collision occurred following stimulation of the lumbar electrode positioned in the ventromedial funiculus, which suggests that the direct descending volleys travel in the ventromedial reticulospinal tracts. In Fig. 5*B* and *C* the segmental delay can be measured between the arriving volley and the beginning

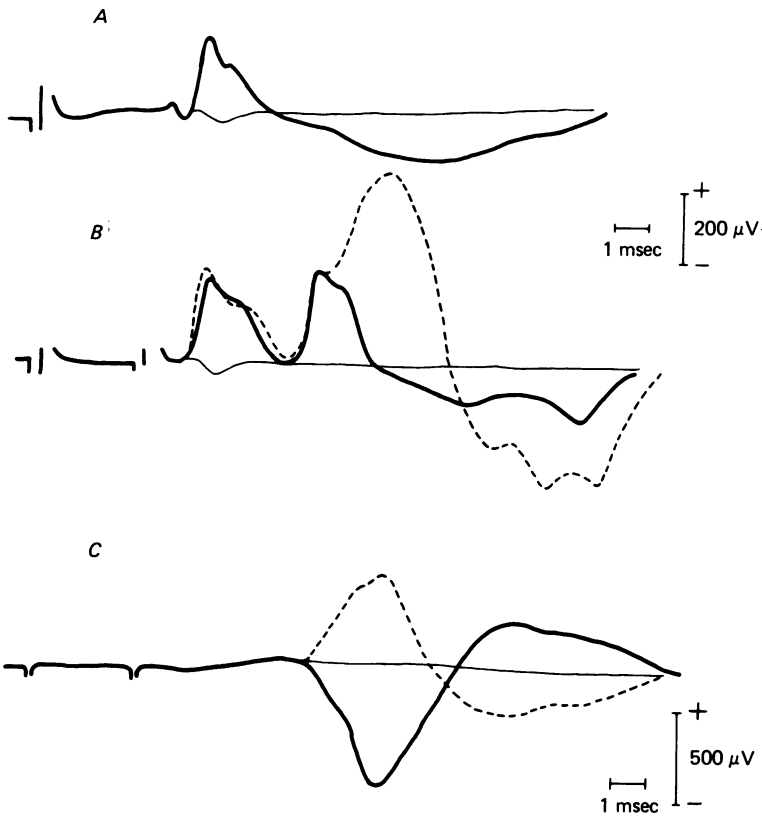


Fig. 6. Computer averaged responses recorded in two external anal sphincter motoneurones following brain stem stimulation. *A* and *B* are from the same motoneurone, *C* from a different motoneurone. *A*, the response obtained upon single shock stimulation. *B*, shows the response obtained upon double shock stimulation. The interrupted line indicates the double shock at increased stimulation strength. *C*, inhibitory response recorded in an external anal sphincter motoneurone upon double shock stimulation. The cell in *C* was slightly depolarized following injection of depolarizing current into the cell. The interrupted line in *C* is the reversed i.p.s.p. obtained following injection of hyperpolarizing current into the cell. The thin straight lines in *A*, *B*, *C* are the extracellular fields. Resting potential for the motoneurone in *A* and *B*: 60 mV. For the motoneurone in *C*: 70 mV.

of the e.p.s.p. This short segmental delay of 0·5–0·6 msec is further evidence that the descending response is monosynaptically evoked.

The histograms in Fig. 7 summarize the properties of the e.p.s.p.s recorded following stimulation of the brain stem reticular formation. It can be seen that short latency, excitatory responses were evoked following ipsilateral and midline stimulation. Stimulation of the contralateral side produced longer latency responses. The

filled squares (■) represent thirteen cells for which segmental delay and conduction velocities were measured. The segmental delay was measured between the arriving volley at the recording site (see for example Fig. 5*B* and *C*) and the beginning of the e.p.s.p. The conduction velocity was measured in the following way: first collision was performed between the brain stem and lumbar stimulating electrodes, in order to assure that both volleys descended along the same pathway; then the distance

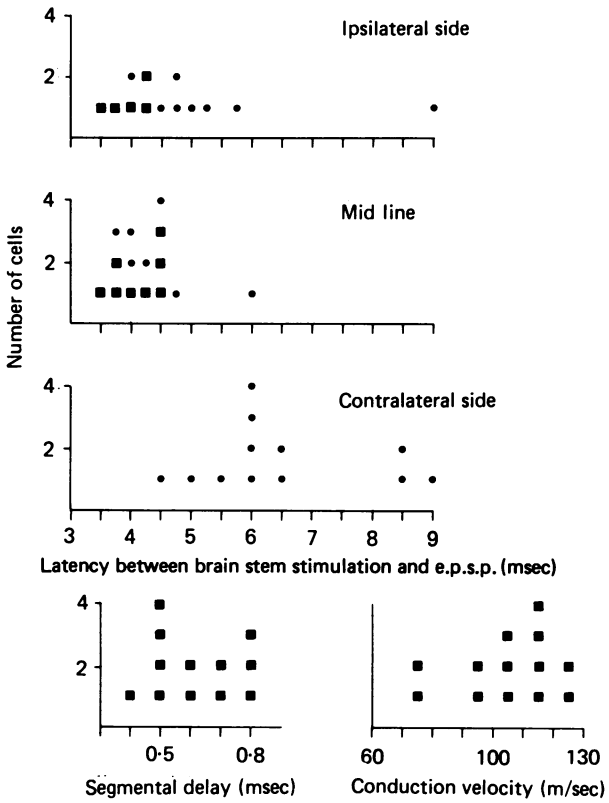


Fig. 7. Latencies, segmental delays and conduction velocities of the e.p.s.p.s obtained following brain stem stimulation. The three upper graphs show the latencies obtained following stimulation of the ipsilateral reticular formation, mid-line and contralateral reticular formation. At the lower left are the segmental delays for thirteen cells (■). At the lower right the conduction velocities of the fibres mediating the responses recorded in the same thirteen cells.

between the two stimulating electrodes was divided by the difference in latency between the response evoked by brain stem stimulation and that evoked by lumbar stimulation. At the bottom left and right of Fig. 7 are the segmental delays and conduction velocities of the selected thirteen cells following ispilateral and mid-line stimulation. The responses recorded in these thirteen cells have short, monosynaptic segmental delays and are mediated by fast conducting fibres.

Fig. 6 gives examples of polysynaptic responses recorded in external anal sphincter motoneurones following brain stem stimulation. Fig. 6*A* and *B* illustrate a polysynaptic component, sitting on the falling phase of the early monosynaptic

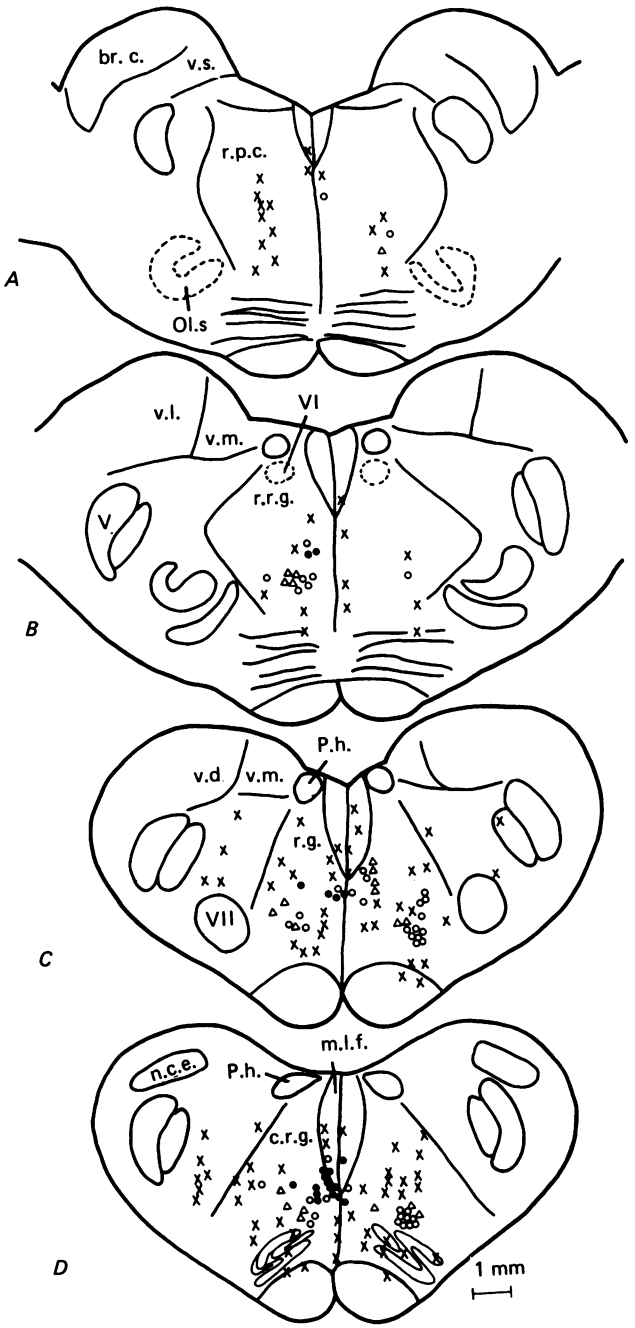
e.p.s.p. This late component displayed significant temporal facilitation when two shocks at higher stimulus strengths were used. Fig. 6*C* shows an example of an i.p.s.p. Inhibition was most readily evoked when double shock stimulation was used. The polysynaptic i.p.s.p.s appear to have been mediated by the ipsilateral ventrolateral reticulospinal tract, because they collided with i.p.s.p.s evoked through the electrode located in the ipsilateral ventrolateral funiculus. No obvious differences in descending effects were observed between the two kinds of sphincter motoneurones.

Brain stem localization of the descending effects

All the stimulus points investigated in the present series of experiments are shown superimposed on a series of schematic cross-sections of the brain stem in Fig. 8. Points from which monosynaptic excitation of sphincter motoneurones could be evoked by a stimulus of 100 μA or less are indicated by a filled circle. Points from which polysynaptic e.p.s.p.s or i.p.s.p.s could be evoked by a stimulus of 300 μA or less are indicated by open circles and triangles respectively. (The higher threshold limit for polysynaptic p.s.p.s was adopted because most of those p.s.p.s had thresholds between 100 and 300 μA, possibly reflecting the need for spatial facilitation within the polysynaptic pathway.) Points from which no responses could be evoked even with a 300 μA stimulus are indicated by a cross.

Since the distributions of effective points for evoking responses of external urethral and external anal sphincter motoneurones were similar, the two distributions have been merged in Fig. 8. Stimulation points from which monosynaptic e.p.s.p.s could be evoked were concentrated at the bottom of the medial longitudinal fasciculus (m.l.f.) and in the ventromedial reticular formation. The m.l.f. points were only found in the caudal part of the nucleus reticularis gigantocellularis (r.g.). In the rostral r.g. the effective points appeared ventrally to the m.l.f. They were not present in the nucleus reticularis pontis caudalis (r.p.c.). Stimulation points evoking polysynaptic e.p.s.p.s were more widespread. Interesting to note was a concentration of stimulation points producing polysynaptic e.p.s.p.s within the contralateral ventrolateral reticular formation. Stimulation of this area has been reported to produce an increase in the e.m.g. activity in the external urethral sphincter (Kuru *et al.* 1963). These contralateral stimulation points are mainly located in the middle and more caudal part of r.g. The most effective stimulation points for causing inhibitory effects were located in the ipsilateral ventrolateral reticular formation. They could also be followed into the rostral r.g.

Fig. 8. Sections through four levels of the ponto-medullary reticular formation showing the effective stimulation points and the points from which no responses could be evoked. *A*, section through r.p.c. *B*, section through the rostral r.g. *C*, section through the middle of r.g. *D*, section through the caudal r.g. According to Brodal's landmarks (1957) lines are drawn on each side to delineate the r.p.c. and the r.g. Abbreviations: V, VI, VII, cranial nuclei. Br.c., superior cerebellar peduncle (bracchium conjunctivum). M.l.f., medial longitudinal fasciculus. N.c.e., external cuneate nucleus. Ol.s., superior olive. V.m., medial vestibular nucleus. V.d., descending vestibular nucleus. V.l., lateral vestibular nucleus.



V.s., superior vestibular nucleus. P.h., perihypoglossal nucleus. c.r.g., caudal nucleus reticularis gigantocellularis. r.g., nucleus reticularis gigantocellularis. r.r.g., rostral nucleus reticularis gigantocellularis. r.p.c., nucleus reticularis pontis caudalis. Symbols: ●, monosynaptic e.p.s.p.s; ○, polysynaptic e.p.s.p.s; △, polysynaptic i.p.s.p.s. ×, no response.

DISCUSSION

Location of the motoneurones innervating the external urethral and external anal sphincters

The location of pudendal motoneurones in the ventrolateral horn of the sacral spinal cord is in agreement with anatomical studies which localized the cell bodies of the pudendal nerve fibres with the chromatolytic (Romanes, 1951; Oliver, Bradley & Fletcher, 1970) or the Horseradish-Peroxidase technique (Sato, Mizuno & Konishi, 1978; **E. Jankowska, Y. Padel & P. Zarzecki, unpublished).**

The pudendal motoneuronal cell group is clearly separated from the other two major cell groups in the sacral spinal cord, namely those motoneurones innervating the tail muscles, which are located ventromedially to the pudendal motoneurones (E. Jankowska *et al.* unpublished) and those parasympathetic motoneurones which have their axons in the pelvic nerve and which are located dorsally to the pudendal motoneurones, in the intermediolateral cell column (Nadelhaft, Morgan, Schauble & De Groat, 1977; Sato *et al.* 1978). This clear separation of the three major motoneuronal cell groups in the sacral spinal cord makes it likely that they get quite different patterns of afferent input.

Segmental reflex connexions

Monosynaptic e.p.s.p.s evoked in sphincter motoneurones by dorsal root stimulation typically had amplitudes less than 1·0 mV while those evoked by peripheral nerve stimulation rarely exceeded 0·5 mV. Furthermore, less than half of the sphincter motoneurones showed monosynaptic responses following stimulation of the peripheral nerve branch containing the motor axon. Thus while monosynaptic reflex connexions to external urethral and external anal sphincter motoneurones exist, they are relatively weak. This is in contrast to hind limb or tail motoneurones, which essentially all receive monosynaptic connexions, usually bigger than 3·0 mV (Eccles *et al.* 1957*a*, *b*; Curtis, Krnjevic & Miledi, 1958; Eccles, 1964; Mendell & Henneman, 1971; E. Jankowska *et al.* unpublished).

The monosynaptic afferents most likely originate in the muscle spindles of the external urethral and external anal sphincters, because the recorded responses were evoked at low threshold and were mediated by rapidly conducting fibres. It is known from the work done in the hind limbs that monosynaptic responses only originate in the muscle spindles and are mediated by low threshold, rapidly conducting afferents (Lloyd, 1943; Eccles *et al.* 1957*a*, *b*).

The existence of monosynaptic reflex connexions of external urethral and external anal sphincter motoneurones suggests that a monosynaptic reflex, similar to that present in other somatic muscles, contributes to the stretch-evoked contraction of the sphincter muscles following their distension (Bishop *et al.* 1956; Garry *et al.* 1959). A possible functional role for such segmental reflex connexions, for instance of the cat external urethral sphincter, could be to aid in preventing the emptying of the bladder.

Duration of the spike after-hyperpolarization

As the sphincter muscles have been described to be of the slow-twitch, tonic type (Bishop *et al.* 1956; Garry *et al.* 1959; Todd, 1964) one would expect a long lasting spike after-hyperpolarization (Eccles *et al.* 1958; Kuno, 1959). The duration of the spike after-hyperpolarization recorded in the sphincter motoneurones was relatively short lasting, falling within the range of the medial gastrocnemius, a fast muscle (Eccles *et al.* 1958; Kuno, 1959). M. Kuno (personal communication) also observed such a short lasting after-hyperpolarization in motoneurones innervating the external anal sphincter. Another exception to the generalization derived from observations of hind limb motoneurones was reported for phrenic motoneurones. While these motoneurones innervate the diaphragm, which is considered to be a slow-twitch type muscle, they too have short duration spike after-hyperpolarization (Gill & Kuno, 1963).

Recurrent inhibition

As described under Results, no evidence for recurrent inhibitory effects could be found in motoneurones innervating the external urethral and external anal sphincters. This is in sharp contrast to hind limb motoneurones, in particular tonic motoneurones, many of which receive recurrent inhibition (Eccles *et al.* 1954; Granit *et al.* 1957; Eccles *et al.* 1961). Apart from sphincter motoneurones, phrenic motoneurones have also been reported free from recurrent inhibition (Gill & Kuno, 1963), while motoneurones innervating muscles moving the head only occasionally receive recurrent inhibition (Rapoport, 1978).

It thus seems that motoneurones innervating muscles which have no obvious antagonists, like the external urethral sphincter, external anal sphincter or diaphragm do not receive recurrent inhibition. It is possible that recurrent inhibition is only found in motoneurones displaying Ia antagonism, as is the case in the hind limb (Eccles *et al.* 1954, 1961), tail (E. Jankowska *et al.* unpublished) and forelimbs (Thomas & Wilson, 1967).

Descending effects

The descending monosynaptic excitation of sphincter motoneurones appears to have been mediated by fast conducting fibres in the ventromedial reticulospinal tract. Other pathways descending in the ipsilateral ventromedial funiculus are the lateral vestibulospinal tract (l.v.s.t.) and the interstitiospinal tract. The participation of the l.v.s.t. in mediating direct descending effects to the sphincter motoneurones can be excluded because many stimulation points in the area of the ventrolateral reticular formation, where the tract descends (Petras, 1967) gave negative results. Stimulus spread to the tract can also be excluded because the stimuli evoking monosynaptic e.p.s.p.s in the present experiments were applied bipolarly and restricted to 100 μA. Measurements of stimulus spread have shown that a 100 μA bipolar stimulus spreads less than 1·0 mm (Bagshaw & Evans, 1976). If one adopts this value as an estimate of the range of stimulus action, one can conclude that the activation of neural elements mediating direct, monosynaptic excitation to sphincter motoneurones are restricted to the r.g. of the medullary reticular formation. A contribution of the interstitiospinal tract can be excluded because stimulation of the m.l.f. rostral to r.g. gave negative results. Such stimulation would activate the interstitio-

spinal tract which originates in the mesencephalon and descends through the pons and medulla in the m.l.f. (Carpenter, Harrison & Peter, 1970).

The ventromedial reticulospinal tract has previously been shown by Grillner & Lund (1968) and Pitts, Fukushima & Peterson (1977) to have a direct excitatory action on hind limb flexor and extensor motoneurones. Although the direct projections to hind limb and sphincter muscles travel in the medial reticulospinal tract, they differ in that the projection to the hind limbs originates from a wider area of the pontomedullary reticular formation than the projection to the sphincters.

The polysynaptic inhibition to sphincter motoneurones resembles that observed in hind limb flexor and extensor motoneurones (Jankowska, Lund, Lundberg & Pompeiano, 1968). This inhibition originates mainly in the ipsilateral ventrolateral medullary reticular formation and appears to descend in the ventrolateral reticulospinal tract. It is unlikely that structures other than reticulospinal pathways mediated the polysynaptic inhibition and excitation observed in the present experiments, because stimulation of points outside the r.g. gave negative results. Stimulus spread to other structures outside the r.g. is also unlikely because the polysynaptic effects were occasionally produced at stimulus strengths as low as 100 μA.

A possible functional role of excitatory reticulospinal input to sphincter motoneurones might be to prevent incontinence by causing co-contraction of sphincter muscles during muscular activity (e.g. coughing, straining, jumping) that might cause an increase in intra-abdominal pressure. The descending effects most likely also play a role in the voluntary control of contracting or relaxing the sphincter muscles. It is very likely that they can at any time interrupt or modulate the segmental reflex actions. Voluntary commands to the sphincter muscles probably leave higher brain structures and are relayed in the brain stem reticular formation. A goal of future experiments will be to localize the origin of the commands and the way they impinge on the relay station. In addition, the functional meaning of the direct and indirect connexions to the sphincter motoneurones remains to be established.

The author wishes to thank Dr B. W. Peterson for valuable advice and comments on the manuscript. This work was supported, in part, by grants NS 02619, EY 02249, and NSF BMS TS 00487.

Note added in proof. After this manuscript was submitted, the work by E. Jankowska *et al.* has been published (Jankowska, E., Padel, Y. & Zarzecki, P. (1978). Crossed disynaptic inhibition of sacral motoneurones. *J. Physiol.* **285**, 425–444).

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