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SPINAL NEURONES WITH LONG PROJECTIONS ACTIVATED FROM THE ABDOMINAL VISCERA OF THE CAT

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SUMMARY

1. Recordings have been made from seventy-three neurones responding to electrical stimulation of pelvic, hypogastric or lumbar colonic nerves, in decerebrate or anaesthetized cats. Fifty-two of the units had long projections that ascended to the first cervical segment, and no units with visceral inputs were found to belong to the spino-cervical tract. Twenty-one units had long descending projections.

2. Twenty percent (i.e. 11/46) responded to parasympathetic (pelvic) nerve stimulation (group 1) whilst 80% (35/46) responded to stimulation of hypogastric and/or lumbar colonic nerves (group 2). Ninety percent of group 2 neurones also responded to pelvic nerve stimulation.

3. The electrical thresholds for activation of the units indicated that the largest peripheral nerve fibres responsible for the response were of the Aδ size.

4. Thirty-one of the neurones had visceral mechanosensitive receptive fields; twenty-one had simple receptive fields in the bladder (seven) or in the colon (fourteen), ten units had compound receptive fields. The response of units with simple receptive fields to mechanical stimulation were either inhibitory or excitatory, and slowly adapting or rapidly adapting. Forty-two units appeared to have no visceral mechanosensitive receptive fields in spite of showing responses to visceral nerve stimulation.

5. Fifty percent of the units tested responded to innocuous somatic stimuli, mostly derived from muscle or joint receptors. Some of the units were found to respond to injections of bradykinin (10–15 μg) into a hindlimb artery.

6. Group 1 had predominantly inhibitory visceral receptive fields, and somatic receptive fields in structures innervated from sacral segments of the spinal cord. Group 2 units all received inputs from visceral nerves entering the spinal cord over lumbar segments; many also received projections from sacral segmental inputs. These inputs showed an equal mixture of excitatory and inhibitory visceral receptive fields and convergence from somatic inputs arising from lumbar as well as sacral dermatomes. It seems likely that this group represents units originating in lumbar as well as sacral segments of the cord.

7. The possible role of these neurones as mediators of visceral sensations and visceral reflexes is discussed.

INTRODUCTION

The micturition reflex and some other visceral reflexes appear to utilize a long ascending spinal pathway (Barrington, 1921; de Groat, 1975) and sensory information from the abdominal viscera is appreciated by man and probably also by other animals, especially those exhibiting voluntary control of micturition or defaecation. There is however, relatively little information regarding the properties of these pathways.

The aims of this study were to record from spinal neurones with long projections that were activated from the abdominal viscera and to determine their properties, particularly their responses to mechanical stimulation of the viscera and electrical stimulation of different visceral nerves.

The possible role of these neurones as mediators of visceral reflexes or visceral sensations is discussed.

METHODS

Experiments were performed on fifty-one cats, of either sex, in the weight range 1·4–5·1 kg. These animals also provided the data described in McMahon & Morrison (1982*a*). Anaesthesia was induced with Fluothane (Halothane, I.C.I.) and animals were either decerebrated at a mid-collicular level ($n = 15$), or anaesthesia was maintained by one of two methods: (i) I.V. administration of 60 mg/kg of alpha-chloralose (BDH Chemicals Ltd., Poole) ($n = 31$); or (ii) by I.V. administration of 35 mg/kg of thiopentone (Intraval sodium, May & Baker) ($n = 5$). Small supplementary doses of anaesthetic (10–30 mg) were given so as to maintain an approximately constant depth of anaesthesia. Animals were ventilated with a positive pressure pump (Starling 'Ideal' pump), and end-tidal CO_2 percentage monitored with an infra-red CO_2 analyser (Model 901, ADC Ltd., Herts.) and maintained between 3·5 and 4·5% by altering stroke volume or rate of respiration.

Carotid arterial blood pressure was monitored with a strain gauge pressure transducer and mean arterial pressure was maintained above 70 mmHg with small infusions (5–10 ml.) of Dextran. Oesophageal temperature was monitored and maintained between 36·5 and 38·5 °C by passing water from a thermostatically controlled water bath through a series of metal tubes situated beneath the animal.

The pelvic cavity was exposed via a low abdominal mid line incision and splitting and retraction of the pubic symphysis. Light-weight stimulating electrodes were then placed around both the left and right pelvic nerve trunks, proximal to the point where the nerves divide to give branches to the colon and bladder. A diagrammatic summary to the innervation of the pelvic viscera is given in Floyd, McMahon & Morrison (1982).

The nerve trunk at this level is unlikely to be contaminated with hypogastric nerve fibres (Crouch, 1969). Stimulating electrodes were also placed around one, normally the left, pudendal nerve. In some experiments all of the electrodes were embedded in a silicone rubber (Silgel 604, Whacker Chemicals GmbH, Munich, Germany), as described by Donoghue, Fox & Kidd (1977).

The urinary bladder was then cannulated with a polythene or nylon cannula, inserted into the viscus via the anterior urethra. In order that access could be gained to the viscera while micro-electrode recordings were made from the dorsal side of the animal, a lateral laporotomy was performed, extending rostrally as far as the last rib and caudally to the ileopectineal arches. The abdominal wall muscles were divided layer by layer. Stimulating electrodes were then placed around both hypogastric nerves about 1 cm from their origin in the inferior mesenteric ganglia, and also around the lumbar colonic nerves and associated inferior mesenteric artery, about midway between the mesenteric border of the colon and the inferior mesenteric ganglia. The electrodes were supported by stitching their leads to the abdominal wall. Inflatable balloons, connected to nylon cannulae, were then introduced into both proximal and distal colon via an incision made in the anti-mesenteric border of the bowel, approximately opposite the inferior mesenteric artery.

A laminectomy of the first three cervical vertebrae was then performed. In all experiments a second laminectomy was performed either (a) of vertebrae T12 to L1, or (b) of vertebrae L5 to L7. The animals were then rigidly fixed into a spinal frame with clamps above and below the exposed spinal cord. The activity of spinal neurones was recorded from the white matter of cervical and thoraco-lumbar cord segments, and the white and grey matter of sacral cord segments, with tungsten micro-electrodes. The electrodes were made by etching short lengths of tungsten wire to produce fine tips. The electrodes were then lowered tip uppermost into a liquid varnish (Type E33, Insl-X Products Ltd., NY) and slowly withdrawn and left to dry. This process produces an insulating layer along the length of the electrode except at the very tip. The most satisfactory electrodes had tip diameters of less than 2 μm and electrical impedances of 400 kΩ at 1 kHz.

The spinal cord was searched for neurones which responded to supramaximal stimulation of all visceral nerves. The stimuli consisted of single shocks or short trains of pulses (4 shocks in 20 msec) at 10 mA, 1 msec duration, repeated once every 2 sec. The recording electrodes were connected to a cathode follower (Model P16, Grass instruments Inc., U.S.A.) with filters set at 100 or 30 μsec rise time (low pass) and 1 msec time constant (high pass). All units found were then tested to ascertain if they possessed a long unbroken projection between the recording site and (1) C1 (in the case of the lumbo-sacral and thoraco-lumbar recordings) or (2) T12 (in the case of cervical recordings). Units with such a projection were selected for study. An unbroken connection was indicated if electrical stimulation of the spinal cord evoked constant latency (less than 0·1 msec variability) spikes which followed high frequencies of stimulation. Attempts were then made to collide the non-synaptically evoked spikes with spontaneous or peripherally evoked spikes (a method similar to that used by Harding & Leek, 1969). On the basis of the outcome of this test, units were designated ascending or descending. Responses of units to electrical stimulation of visceral nerves and to mechanical stimulation of visceral structures (manual manipulation, discrete probing, distension of the bladder or colon, or spontaneous contractions) or somatic stimulations were recorded, along with visceral pressures on an FM tape recorder (Thermionic T3000). Units not responding to any of these forms of mechanical stimulation of the viscera were classified as not possessing a visceral mechanosensitive receptive field. In some cases units were tested for responses to injections of small quantities (10 or 15 μg) of bradykinin into a hind limb artery. The locations of units within the spinal cord were estimated by plotting the depths and mediolateral coordinates of units (from micromanipulator readings) on to maps of the spinal cord constructed from transverse sections of the cord post mortem.

Identification of axonal vs. somatic spikes

In this study the majority of neurones with long ascending axons were recorded in the lateral funiculus and had spike shapes characteristic of axons (Hunt & Kuno, 1959). A small number of units with long ascending projections were recorded from the grey matter of the sacral end and their spike shapes suggested that their recordings were from cell bodies.

Analysis of evoked responses

Post-stimulus time histograms were obtained following the electrical stimulation of afferent nerves. For units with background firing mixed excitatory and inhibitory responses could be seen and the latencies, magnitudes and durations of inhibitory or excitatory responses were calculated as follows.

A horizontal line was drawn across the post-stimulus time histogram at the level of the number of spikes/bin occurring during the phase when the effects of the stimulus were completed. This level is determined by the background firing of the unit. An example of such a line is shown in Fig. 3 as $x-x_1$. The latency and duration of the response were judged as the time when the histograms deviated from this line and the time taken from the histogram to re-approach the line respectively. The magnitude of the response was judged in different ways for excitatory and inhibitory responses. For the former, the area above the line was calculated, and this area (a particular number of spikes) divided by the number of stimuli. In this way excitatory responses were determined as the number of spikes/stimulus. For inhibitory responses the average number of spikes/bin during the deviation of the histogram was calculated. A percentage inhibition was then determined as

$$100-\frac{(\text{average bin value during response})\times 100}{(\text{average bin value due to background firing})}.$$

RESULTS

Recordings have been made from seventy-three units which responded to stimulation of visceral nerves and which also possessed a long unbroken projection reaching at least as far as the third cervical segment. The collision test revealed that for fifty-two of these units the projection was ascending, while for the remaining twenty-one units the projection was descending. All of the ascending axons were also found to project to C1, i.e. it is unlikely that any of the units belonged to the spino-cervical tract; this conclusion is in agreement with the observations of Cervero & Iggo (1978) that no spino-cervical tract cells exhibited bladder inputs.

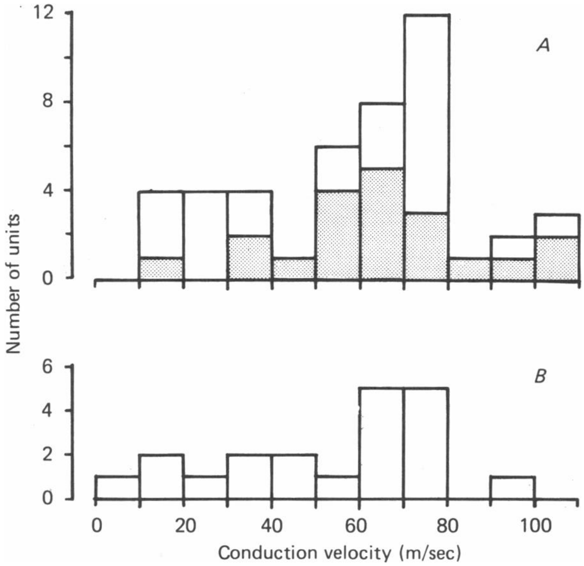

Fig. 1. Histograms of the estimated conduction velocities in the long ascending units (*A*), and the descending units (*B*). Stippled areas in *A* refer to ascending units which possessed vesical mechanosensitive fields.

Conduction velocities

The collision test also permitted an estimate of the conduction velocity of the unit to be made. Histograms of calculated conduction velocities of ascending and descending units are shown in Fig. 1. The range of conduction velocities was 6·7 m/sec to 103 m/sec, with a mean value of 58 m/sec. The histogram for ascending units shows peaks at 10–30 m/sec, 70–80 m/sec and possibly 100–110 m/sec. The ascending units are divided into those with and those without visceral mechanosensitive receptive fields. The peak at 10–30 m/sec is largely composed of units which did not have visceral mechanosensitive fields.

The descending units have a somewhat different distribution, with conduction velocities ranging from 6·7–75 m/sec. The histogram of conduction velocities, shown in Fig. 1 *B*, shows only one peak, occurring between 60 and 80 m/sec. Below 60 m/sec conduction velocities were fairly evenly distributed.

Location of units

Locations of the units within the spinal cord, at the level of the recording electrode, are shown in the composite maps of Fig. 2. As can be seen, the long ascending fibres recorded in the cervical segments tended to lie in the intermediolateral and ventrolateral aspects of the lateral columns. The long ascending units recorded in the sacral cord appeared to be located in Rexed's laminae IV and VI, with the exception of one unit which was located in the ventral white matter.

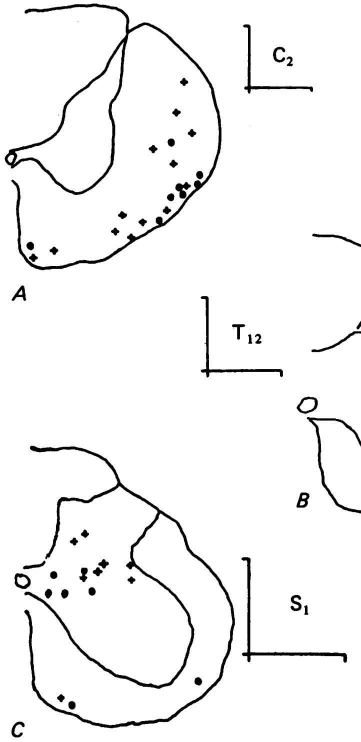

Fig. 2. Locations of sixty-four units recorded within the spinal cord at (*A*) the second cervical segment, (*B*) the twelfth thoracic segment, and (*C*) the first sacral segment. Calibration bars represent 1 mm. Filled circles indicate the location of descending units, and crosses the location of the long ascending units.

Descending units in the cervical cord occurred predominantly in the ventrolateral column but with one unit situated dorsally in the intermediolateral column and one unit in the ventral white matter. In the thoracic cord the distribution was, as for the long ascending units, shifted dorsally; units were reasonably tightly grouped in the dorsolateral column but with two units in ventral white matter.

Resting activity of units

Many units (thirty-eight out of fifty-two ascending units, and thirteen of twenty-one descending units) exhibited some firing in the apparent absence of stimulation, i.e. with the viscera empty and the animal immobilized. In all but one case the resting discharge rate appeared to be stationary, i.e. the mean rate did not change with time. The anomalous unit exhibited a discharge rate which varied cyclically between 0 and 40 spikes/sec, with a period of 15–60 sec. Interspike interval histograms for resting discharge could be uni- or multi-modal, but in nearly all cases the trailing edges of the distribution were smooth and apparently exponential. An explanation consistent with this form of interval distribution is that the spike train results from the superposition of several or many synaptic inputs (Holden, 1976). In this case the dominant input produces the modal value.

Units without visceral mechanosensitive receptive fields tended to have lower discharge rates (mean = 5·1 spikes/sec) than units with visceral mechanosensitive receptive fields (mean = 12·3 spikes/sec); (statistically significant at the 5% level using a Student's *t* test), and this group also exhibited a higher proportion of units with no resting discharge (twenty out of forty-two compared with two out of thirty-one).

Effects of anaesthesia on background firing

Even when consideration was given for the association between low firing rate and lack of visceral receptive fields, there were clear differences in the mean resting spike rates in different preparations; decerebrate cats generally exhibited higher resting rates than anaesthetized preparations.

Response of units to electrical stimulation of peripheral visceral nerves

The response of seventy-three neurones to supramaximal stimulation of pelvic, hypogastric and lumbar colonic nerves was tested. In order to measure the latency and magnitude of evoked responses, post-stimulus time histograms were constructed using bin-widths of 2 or 20 msec. An example of a typical response and derived stimulus time histograms is shown in Fig. 3.

Convergence from different visceral nerves

Most of the units fully tested showed inputs from more than one visceral nerve (forty-four of our sixty-four). The remaining twenty units showed inputs from only one visceral nerve (pelvic, hypogastric or lumbar colonic). Table 1 shows the number of units exhibiting different combinations of inputs. Of the units receiving only one input, this input was most commonly the pelvic nerve (fifteen out of twenty cases). None of the units was activated solely by lumbar colonic nerve stimulation.

Nature of responses to electrical stimulation

For units with no background activity only excitatory responses could be detected. For units possessing background activity, primary responses could be either excitatory or inhibitory. For the latter group various patterns of response were seen, viz. excitation only, excitation followed by inhibition, excitation followed by inhibition followed by excitation, excitation followed by secondary excitation (after some interval), or excitation followed by inhibition followed by excitation followed by inhibition. For the group exhibiting inhibitory primary responses fewer combinations were observed, viz. inhibition alone, inhibition followed by excitation, inhibition followed by excitation followed by inhibition. One of the most striking features to emerge was that when the units showed convergence from two or three visceral nerves, the pattern of excitation and/or inhibition following electrical stimulation of any nerve was identical in all but three out of forty-four cases studied.

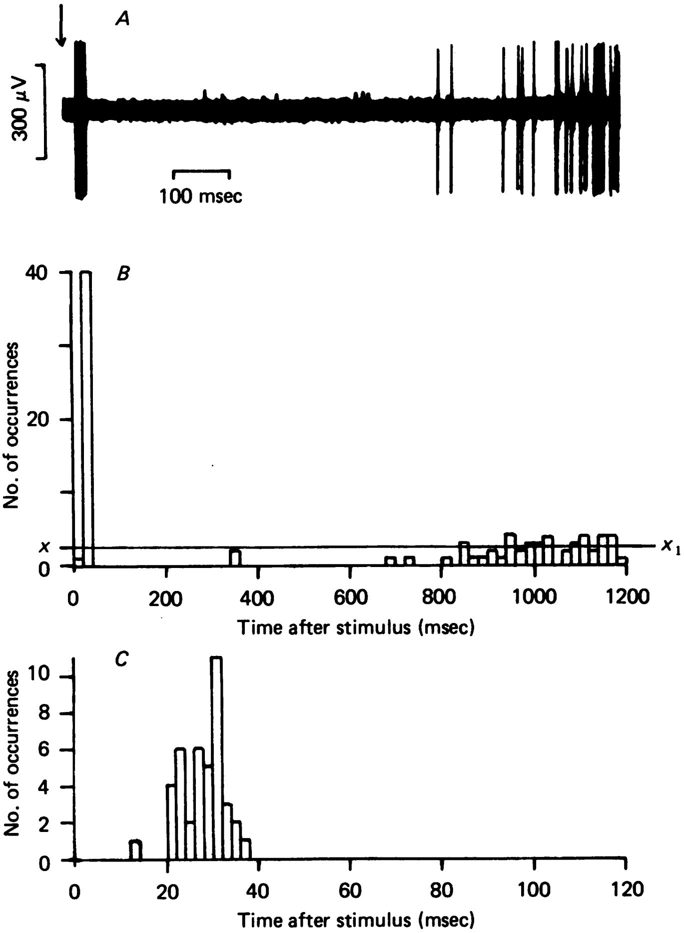

Fig. 3. *A*, ten superimposed responses of a long ascending unit to electrical stimulation of the pelvic nerves (indicated by the arrow). *B* and *C*, post-stimulus time histograms constructed from a series of sixty evoked responses. Bin width: 20 msec (*B*) and 2 msec (*C*).

Primary responses

Table 2 shows the latencies, magnitudes and durations of excitatory and inhibitory evoked responses for ascending and descending units divided according to the nerve stimulated. There were no obvious differences between units with and without visceral receptive fields. Latencies of response for descending units were, on average, significantly longer than for ascending units ($P < 0.05$). The average magnitude and duration of both excitatory and inhibitory responses were very similar for individual nerves.

Secondary responses

Secondary excitatory responses had an average latency of 82 msec, duration 42 msec, and magnitude 0·8 impulses per stimulus. Secondary inhibitory responses had an average latency of 50 msec, duration 190 msec, and magnitude 80% inhibition.

TABLE 1. Patterns of convergence from peripheral visceral nerves. The proportion of units receiving inputs from one, two or three visceral nerves is shown. Units responding to only a single nerve have been divided according to which nerve was effective (PN, pelvic nerve; HGN, hypogastric nerve; LCN, lumbar colonic nerves)

	Single nerve			Convergence	
	PN	HGN	LCN	two nerves	three nerves
Long ascending	11/46	4/46	0/46	15/46	16/46
Descending	4/18	1/18	0/18	9/18	4/18

TABLE 2. The latencies, magnitudes and durations of primary excitatory and inhibitory responses to visceral nerve stimulation, divided according to the level of the recording electrode and the nerve stimulated. Abbreviations as in Table 1

	Long ascending units			Descending units		
	PN	HGN	LCN	PN	HGN	LCN
Latency of excitatory response (msec)	21	15	14	54	37	40
Latency of inhibitory response (msec)	16	12	20	29	32	27
Magnitude of excitatory response (spikes/stimulus)	1·35	1·35	1·45	1·55	1·10	0·14
Magnitude of inhibitory response (% inhibition)	87	80	95	89	50	87
Duration of excitatory response (msec)	22	24	23	23	17	10
Duration of inhibitory response (msec)	58	50	90	100	110	100

Responses from the pudendal nerve

Twelve ascending and nine descending units were tested for their responses to pudendal nerve stimulation. Nine of the ascending and all of the descending units responded to such stimulation. The pattern of responses to pudendal nerve stimulation, in nearly all cases, did not deviate from that seen following visceral nerve stimulation. All of the units responding to stimulation of the pudendal nerve responded to pelvic nerve stimulation, and twelve of these showed convergence from sympathetic nerves. The latencies of evoked response from the pelvic and pudendal nerves were very similar and when these latencies were plotted against each other, for individual units, a very good positive correlation was found to exist (correlation coefficient = 0·96, intercept not significantly different from zero). This similarity between pudendal and pelvic nerve inputs may simply reflect the homogeneity of the cord segments of termination of the afferents in these nerves.

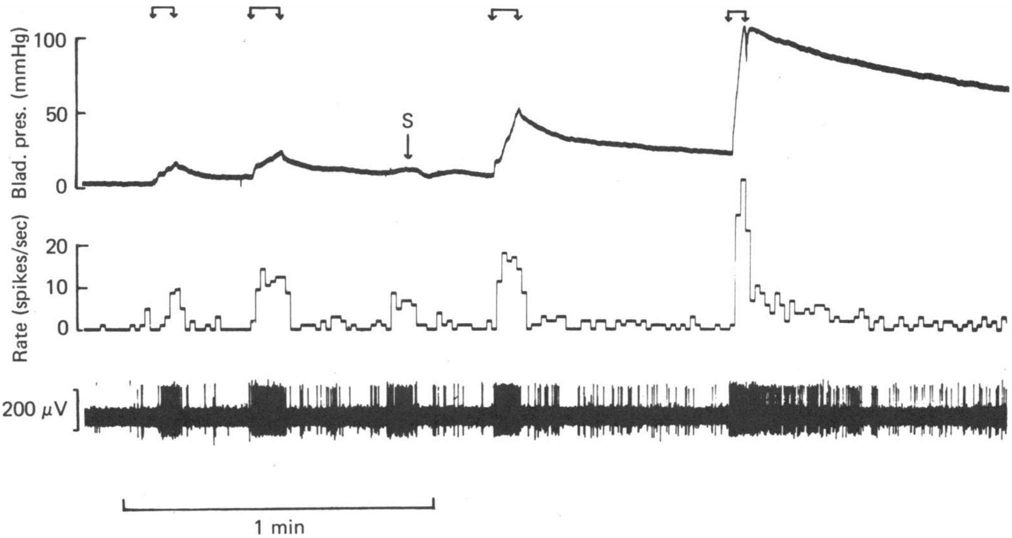

Fig. 4. The response of a long ascending unit (bottom) to a series of bladder distensions to different pressures (top). The middle trace indicates spike rate counted in bins of 1 sec. At each set of arrows 10 ml. warm saline was injected into the bladder, and the effect of a small spontaneous bladder contraction can be seen at S.

Electrical threshold

The strength of the stimulus (in milliamps) at which responses were first seen was determined in many cases ($n = 37$). The average threshold for visceral nerves was $1{\cdot}7 \pm 0{\cdot}18$ mA when single shocks were employed, and $1{\cdot}5 \pm 0{\cdot}10$ mA when a short tetanus of four shocks was used. There were no significant differences between thresholds of individual visceral nerves. Average pudendal nerve thresholds were $1{\cdot}0 \pm 0{\cdot}10$ mA (one shock) and $1{\cdot}0 \pm 0{\cdot}06$ mA (four shocks). (All values are means ± S.E. of means). Pudendal nerve thresholds were significantly lower than the visceral nerve thresholds.

In experiments where mass waves were recorded from visceral nerves or dorsal roots, it was found that the Aδ component of the nerve trunk was activated by stimulus strengths of 1·5 mA above (hypogastric) and 1·6 mA and above (pelvic). C fibre volleys were only detected at strengths in excess of 4·5 mA. It would seem likely that for all of the units examined in this study, the largest diameter visceral afferents affecting the cells were of the Aδ class.

Responses of units to 'natural' stimulation of the abdominal viscera

Thirty-one units (twenty-four ascending and seven descending) were found to possess visceral mechanosensitive receptive fields. Twenty-four excitatory fields and seventeen inhibitory fields were present in the bowel or bladder. Responses to mechanical stimulation could be slowly adapting or rapidly adapting. Examples of such responses are seen in Figs. 4 and 5.

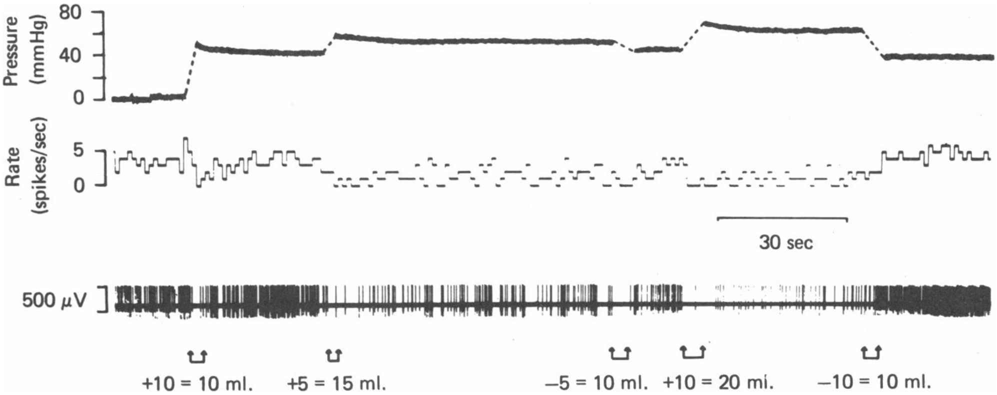

Fig. 5. The response of a long ascending unit (bottom) to distension of the colon to different pressures (top). The middle trace indicates spike rate counted in bins of 1 sec. Between the arrows volumes of warm saline were injected into the lumen of the colon.

Receptive fields could be subdivided according to their size and sign. *Simple* receptive fields were monovisceral and could be excitatory or inhibitory. *Compound* receptive fields were multivisceral and of two types: type I, where fields in different viscera were both excitatory or both inhibitory; and type II where receptive fields in different viscera were of opposite sign. Approximately two thirds of the ascending units in this study had simple receptive fields, half of these being excitatory and half inhibitory. In the remaining units all but one possessed Compound type I receptive fields.

For units exhibiting slowly adapting responses, adapted discharge rates have been determined at various steady state levels of intravesical or intracolonic pressure as shown in Fig. 6.

Different units exhibited curves with different characteristics but the range of patterns shown by the population indicate that the level of distension is monitored over a wide range of pressures. The minimum observed effective pressure for bladder distensions was 10 ± 6 mmHg (mean ± S.D.), whilst for bowel distensions it was 22 ± 13 mmHg (mean ± S.D.).

Rapidly adapting responses showed an exponential-like decay of their discharge rates after the onset of stimulation. The half-time of response for bowel stimulation was 3·0 ± 1·4 sec and for bladder distension 1·6 ± 0·4 sec (mean ± S.D.). The changes in discharge rate during this rapidly adapting, or phasic, response tended to be much larger than the changes in adapted discharge rate seen in slowly adapting responses.

It was possible to construct adapted discharge rate *vs.* pressure curves for pressures reached by both passive distensions and active contraction. An example of such a plot, derived from the responses of one unit, is shown in Fig. 7. As can be seen, pressures reached by active contractions produced greater changes in discharge rate than passively obtained pressures. This difference was seen in all cases where steady-state pressures were obtained by both active contractions and passive distensions.

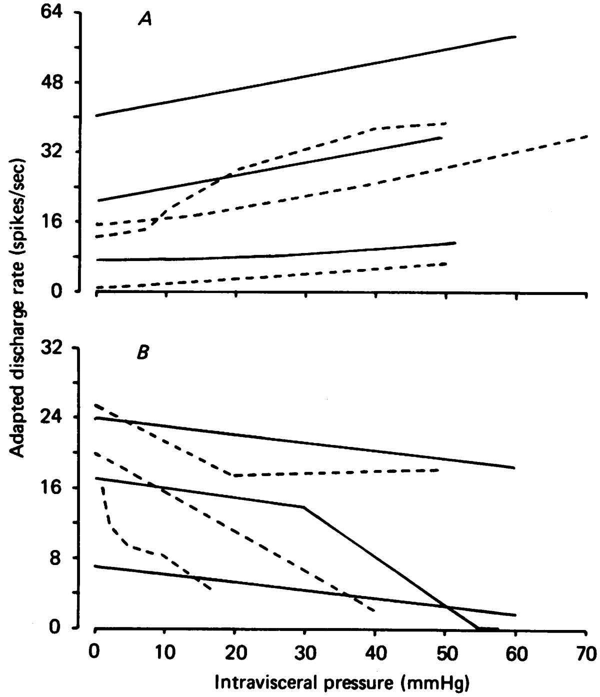

Fig. 6. The relationship between adapted discharge rate and intravesical (dashed lines) or intracolonic (continuous lines) pressure for twelve long ascending units which showed either a positive relationship (*A*) or an inverse relationship (*B*). For clarity error bars have not been shown but in all cases there was a statistically significant difference between points in the lower and higher ranges of intravesical pressure.

In all of the units studied there was no case whatsoever of a unit showing qualitatively different responses (i.e. excitatory and inhibitory) to different forms of mechanical stimulation of one site (i.e. compressions, distension, contractions).

Receptive fields

No attempts were made to delineate the detailed distribution of receptive endings. However, some impression of the size and position of receptive fields was gained by probings and manipulations of the viscera. Receptive fields in the bowel tended to occupy the terminal 5 cm of the colon and rectum and the response to mechanical stimulation depended on stimulus intensity and the area of tissue stimulated.

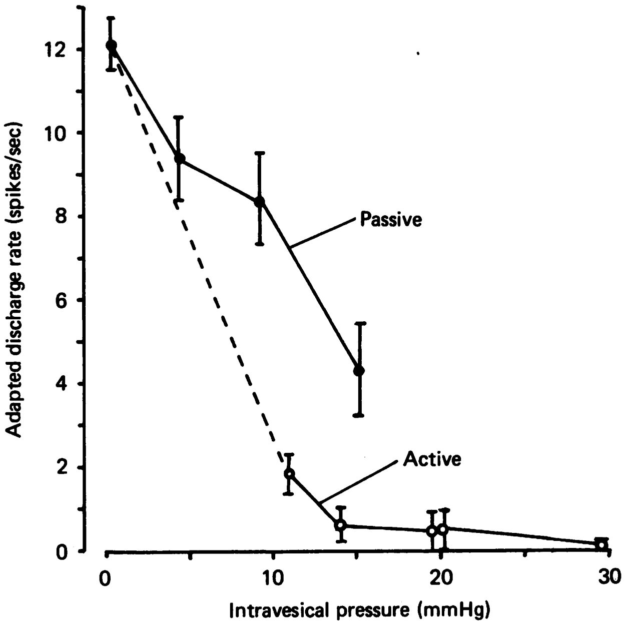

Fig. 7. The inverse relationship between adapted discharge rate and intravesical pressure for one long ascending unit, during passive distension and active contraction of the bladder. Error bars represent 1 S.E. of the mean.

TABLE 3. Types of visceral receptive fields found in different classes of unit. (+) and (−) indicate excitatory and inhibitory fields respectively

	Long ascending	Descending
Number of units with visceral mechanosensitive sites	24	7
Number of units with receptive fields in one viscus only	17	4
Number of units with receptive fields in two viscera	7	3
Number with bladder (+) fields only	4	0
Number with bowel (+) fields only	5	3
Number with bladder (−) fields only	2	1
Number with bowel (−) fields only	6	0
Number with (+) fields in both viscera	4	1
Number with (−) fields in both viscera	2	1
Number with bladder (−) and bowel (+) fields	0	1
Number with bladder (+) and bowel (−) fields	1	0
Total number (+) fields	18	6
Total number (−) fields	13	4

Receptive fields in the bladder were also large and diffuse and sometimes crossed the mid line. Electrical stimulation (i.e. 5 mA, 1 msec pulses) of the bladder wall was often found capable of evoking discharges in the units under study, even in cases where no mechanosensitive field was in evidence.

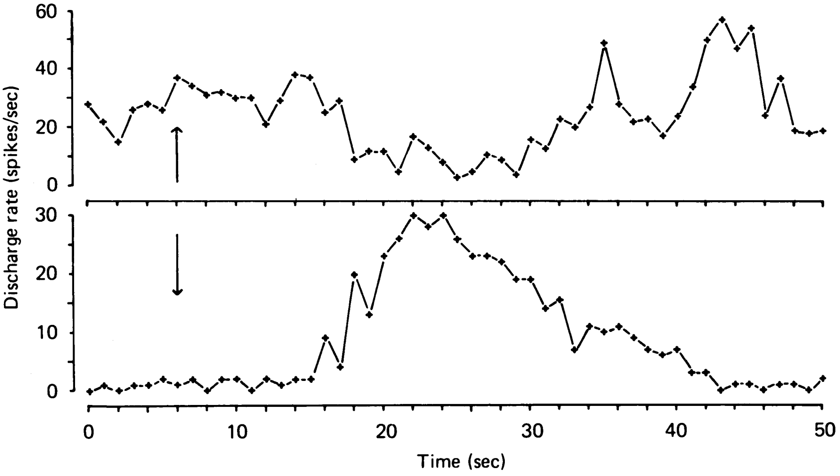

Fig. 8. The response of two long ascending units to intra-arterial injections of bradykinin (10 μg in top record, 15 μg in bottom record).

Distribution of receptive fields

Table 3 shows the numbers of units possessing different types of visceral receptive field. Most of the ascending units which were recorded in the sacral cord (and which presumably originated there) possessed inhibitory receptive fields. Further, these units showed responses to electrical stimulation of only the pelvic nerve, or shorter latencies of response to pelvic nerve stimulation than to sympathetic nerve stimulation (by an average of 5 msec). The remaining ascending units, recorded in thoraco-lumbar white matter (and presumably including units originating from lumbar or sacral segments), exhibited a more equal proportion of excitatory and inhibitory receptive fields.

In order to investigate the tendency for inhibitory fields to be characteristic of sacral neurones and for lumbar neurones to be associated with excitatory fields the units have been divided into two groups: group 1, those responding only to pelvic nerve stimulation, and group 2, those responding to sympathetic stimulation. Nearly all of the group 2 neurones also responded to pelvic nerve stimulation.

Three-quarters of the group 1 cells possessed simple (i.e. monovisceral) inhibitory receptive fields, whereas those of the remainder were multivisceral and 50 % of these fields were also inhibitory. Group 2 cells exhibited a similar ratio of simple to compound receptive fields, but the large majority of simple fields were excitatory. This difference is even more pronounced if one considers the group 2 units without pelvic nerve inputs, where nearly all receptive fields were excitatory.

TABLE 4. The effects of somatic innocuous and noxious stimulation on the long ascending and descending units (BK, bradykinin, 10 or 15 μg; VMRF, visceral mechanosensitive receptive field)

	Long ascending neurones		Long descending neurones	
	With VMRF	No VMRF	With VMRF	No VMRF
Number responding to somatic stimulation	13	9	4	3
Number *not* responding to somatic stimulation	4	14	2	8
Number with receptive fields in hind limb/flank	8	4	4	1
Number with receptive fields in tail/perineal skin	5	5	2	2
Number giving *no* response to bradykinin	1	2	0	3
Number giving increased discharge following BK	0	1	0	0
Number giving decreased discharge following BK	1	1	0	0
Number with somatic field and responding to BK	1	1	0	0
Number *not* responding to BK but with somatic receptive field	1	1	0	0

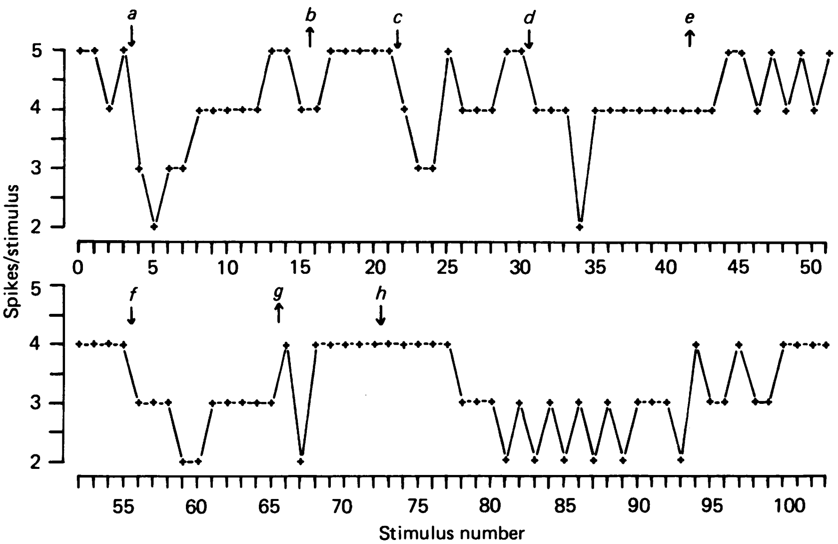

Fig. 9. The response of one long ascending unit to electrical stimulation (10 mA, 1·0 msec pulses at 2 sec intervals) of the pelvic nerve. 20 ml. saline were injected into the colon at *a* and removed at *b*. 20 ml. were again injected into the colon at *c*, followed by a further 10 ml. at *d*, and 30 ml. were removed from the viscus at *e*. Between *f* and *g* toothed forceps were applied to the skin of the contralateral hind limb, and 15 μg bradykin was injected I.A. at *h*.

Somato-visceral convergence

Twenty-nine of fifty-seven units tested responded to innocuous somatic stimulation and excitatory and inhibitory responses were both seen. The magnitude of response was often similar to that of visceral stimulation. Nine of the units were also tested for responses to bradykinin (10 or 15 μg) and Fig. 8 shows the response of two units to such an intervention.

Table 4 collates the information regarding somatic receptive fields, most of which were in muscles or joints. Most of the units in group 1 had somatic receptive fields in the tail/perineal region, whereas group 2 neurones exhibited more equal proportions of receptive fields in the hindlimb and tail.

In some units with no resting discharge, the presence of an inhibitory receptive field could not be directly demonstrated, but would be inferred by considering the effects of visceral or somatic stimuli on responses evoked by nerve stimulation. Fig. 9 shows the effects of somatic and visceral stimuli on one such unit.

DISCUSSION

Anatomical studies (e.g. Haggqvist, 1936) indicate that the majority of fibres in the ventral or lateral columns are small (i.e. less than 4 μm diameter). However, the conduction velocities of units recorded in this experiment were only occasionally less than 20 m/sec (fibres with a conduction velocity of less than 20 m/sec are likely to be less than 4 μm in diameter (Gasser & Grundfest, 1939; Hursh, 1939)). Lalley, de Groat & MacLean (1972) and de Groat (1975) have suggested, on the basis of mass waves recorded in the brain stem that the ascending limb of the micturition reflex may transmit at only 10–11 m/sec. However, in papers by de Groat & Lalley (1972) and Lalley *et al.* (1972), pelvic nerve stimulation produced mass waves in spinal segments S2 and L4 with latencies of 5 and 23 msec respectively and in the brain stem with latencies of 30–40 msec. On this basis the conduction velocity of the pathway between L4 and the brain stem would be about 30 m/sec, which corresponds to the conduction velocity in other autonomic spino-bulbar pathways (Coote & Downman, 1966).

One striking feature of the results is that approximately half the units had no demonstrable visceral mechanosensitive receptive fields. One explanation for this finding is that these units receive input from primary afferent fibres which are not mechanosensitive, e.g. thermoreceptors, or chemoreceptors. There are several reasons why this is unlikely. First there are no reports of such primary afferents in the hypogastric or pelvic nerves (and Nathan (1952) has shown that the body of the bladder in man is thermally insensitive). Secondly, in terms of locations and responses to electrical stimuli, there were no apparent differences between these and units which did have mechanosensitive sites. Units without receptive fields, however, tended to have lower resting firing levels, 50 % of them having no resting discharge. It may be that some of these units received inhibitory inputs which were not revealed because of the absence of resting discharge. Moreover, units with mechanosensitive receptive fields and high levels of resting discharge were more common in non-anaesthetized than in anaesthetized animals.

The ascending units were divided into group 1 units with inputs from the pelvic nerves, but not the hypogastric or lumbar colonic nerves. These units had predominantly inhibitory receptive fields in the viscera, and exhibited somatic convergence from sacral dermatomes. Group 2 units, which were all evoked by afferents in the sympathetic nerves, and often also responded to pelvic nerve stimulation, contained approximately equal proportions of excitatory and inhibitory visceral receptive fields, and showed somatic convergence from sacral and/or lumbar dermatomes.

Possible reflex mechanisms utilizing group 1 and group 2 neurones

Current views on micturition disregard the hypogastric nerves as a source of afferent input and suggest that they have no role to play in bladder reflexes in normal conditions (de Groat 1975). There is no doubt however that the slowly adapting mechanoreceptors in the hypogastric nerve can monitor the physiological state of the bladder, and the functional characteristics are similar to those of the pelvic nerve (Winter, 1971; Floyd, Hick & Morrison, 1976; Floyd & Lawrenson, 1979). In addition, this study has shown that the majority of long ascending units in the spinal cord that receive an input from the pelvic nerve also receive a projection from the hypogastric nerve. This seems at first sight to be incompatible with the results of denervation studies which indicate that the hypogastric afferents are not essential and cannot substitute for the pelvic nerve afferents in initiating the reflex contractions of the bladder seen after raising intravesical volume (Edvardsen, 1968; Gjone, 1965). The dilemma can be resolved however by considering the hypothesis that has evolved as a result of the data presented in this series of papers (Floyd *et al.* 1982; McMahon & Morrison, 1982*a*, *b*): two separate neural mechanisms are active in micturition, (*a*) a series of spino-bulbo-spinal (reflexes that can be elicited from many sources, including the pelvic and hypogastric nerves, and (*b*) a gating mechanism that controls the micturition threshold, and is activated by pelvic, but not by hypogastric, nerve inputs from the bladder (McMahon & Morrison, 1982*b*).

The gating mechanism probably acts by modulating transmission between descending bulbo-spinal pathways and the preganglionic efferents, and may utilize the sacral interneurones without hypogastric nerve inputs described by McMahon & Morrison (1982*a*) that had compound type II receptive fields.

A further effect of this hypothesis would be that there is little need for specificity of projection in the descending pathways. It is of interest that all the descending neurones in this study exhibited widespread convergence; these bulbo-spinal axons may not only influence the pelvic nerve efferents via the gating mechanism, but also end on neurones with other functions.

The case for the involvement of the long ascending units from this study in the spino-bulbo-spinal component of the micturition reflex depends (*a*) on the demonstration (McMahon & Morrison, 1982*b*) that electrical stimulation of the pelvic, hypogastric, lumbar colonic and pudendal nerves can produce efferent firing in pelvic nerves with similar latencies; (*b*) on the demonstration that somato-visceral convergence affects the motility of the bladder (Macpherson, 1966; Sato, Sato, Shimaoa & Torigata, 1975; Sato, Sato & Schmidt, 1979); and (*c*) on revisions of the estimates of the conduction velocities of axons in different parts of the micturition reflex pathway given by Lalley *et al.* (1972) and de Groat (1975). These estimates do not allow for transmission delays at any site in the pathway; delays of 15–25 msec were found in the dorsal horn in this study, and the conduction velocities that would be consistent with a brain stem latency of 30 msec (de Groat, 1975) fall in the range 20–70 m/sec, which corresponds to the conduction velocities of the majority of the units found in this study.

There seems little doubt that a considerable degree of convergence takes place at some stage in the spino-bulbo-spinal pathways that influence the vesical parasympathetic efferent nerves (McMahon & Morrison, 1982*b*). The evidence presented here suggests that much of the convergence has occcured in the spino-bulbar limb of the pathway. However the relationship between the electrically evoked characteristics of the units and their response to mechanical stimulation requires further elucidation; any of the units may contribute to electrically evoked spino-bulbo-spinal reflexes, but only half of them were influenced by mechanical stimulation of the viscera. Two important questions arise from this finding: (*a*) the circumstances in which units without mechanosensitive receptive fields are influenced by visceral events other than the intervention of an electrical stimulus, and (*b*) whether the ascending pathways that evoke a spino-bulbo-spinal reflex in the pelvic nerve respond to natural stimuli. The arguments presented earlier suggest that the units may be mechanosensitive when released from descending controlling influences. If the ascending limb of the spino-bulbo-spinal reflex is capable of responding to natural stimuli, then at least two different transfer functions are possible: approximately 50 % of units with receptive fields in the bladder were excited and the remainder were inhibited during periods of raised intravesical pressure. The existence of spinal neurones that give inverse transfer characteristics to a rise in the bladder pressure has been recognized previously (Kamikawa, Matsua, Koshino & Kuru, 1962; Cervero & Iggo, 1978), but they do not feature in current theories of micturition. Statistically, cells showing inverse functions to bladder or colonic pressure dominated in the population of units with pelvic but without hypogastric nerve inputs. In contrast, units with positive response functions formed the majority of cells in the population that received hypogastric, but not pelvic, nerve inputs. The distribution of the somatic receptive fields and the segmental input from the viscera suggest that the former population originated from sacral segments, and the latter from lumbar segments. These two populations however account for only 30 % of the sample, and the majority of cells had inputs from the pelvic and hypogastric nerves, and exhibited excitatory or inhibitory visceral receptive fields and somatovisceral convergence within the lumbo-sacral segments. Reciprocal relationships are a common feature of populations of central neurones thought to be concerned with respiratory or cardiovascular regulation (Burns, 1963; Merrill, 1974), and it may be that reciprocal acitivities may be found in the descending as well as the ascending pathways of the micturition reflex.

The transfer functions of these neurones are of interest because their thresholds correspond to those for reflexes and non-painful sensations that can be elicited from the bladder and colon. The mean pressure threshold for bladder inputs was 10 mmHg which is greater than the micturition threshold and close to the pressures that were reported to elicit sensations of bladder filling by Denny-Brown & Robertson (1933). The mean pressure threshold for the colonic inputs was 22 mmHg which is slightly higher than for sensations described by White, Verlot & Ehrentheil (1940). The thresholds of the ascending neurones were therefore within the range which mediates physiological responses, and the stimulus–response curves for static pressures cover the supraphysiological as well as the normal range of intravisceral pressures. The transfer function for bladder inputs is to the left of that for colonic inputs; both groups may be subdivided into units with either direct or inverse transfer functions. The ability of the latter group to signal the higher range of visceral pressures depends partly on the resting discharge of the cell; no information can be signalled about pressures higher than that which causes complete inhibition of the cell, unless the level of resting discharge is increased.

In some cells which appeared to have no mechanosensitive receptive field, the evoked responses to electrical stimuli could be reduced by raising intravisceral pressures, which suggests that some of these cells would have shown inverse transfer functions if a resting discharge had been present. With regard to the transfer of information concerning noxious stimuli, the thresholds of ascending neurones to static visceral pressures are within the range occurring during normal micturition and colonic reflexes, and the stimulus–response curves of cells with direct transfer functions commonly cover the ranges of intravisceral pressures associated with responses to physiological and supraphysiological stimuli.

The occurrence of convergence from low and high threshold somatic receptors in neurones with visceral inputs in this study confirms the existence of viscero-somatic convergence reported previously by Pomeranz, Wall & Weber (1968), Selzer & Spencer (1969*a*, *b*), Fields, Meyer & Partridge (1970*a*), Fields, Partridge & Winter (1970*b*) and Hancock, Rigamonti & Bryan (1973). Most of these authors have interpreted their results in terms of the convergence projection theory of referred pain (Ruch, 1949), but as many of the inputs to these cells have low thresholds, it may be that functions other than referred pain, such as the somatic and visceral influences on the external sphincters, may be subserved by these neurones.

The properties of neurones with descending projections varied considerably, although the latencies of evoked responses were consistently greater than for other groups. The descending neurones generally exhibited a high degree of convergence (from different visceral nerves) and their responses to mechanical stimulation were generally phasic. The exception to this rule occurred in two units which gave persistent discharges to a brief mechanical stimulus. In some cases the firing outlasted the stimulation by up to 30 sec.

There is certainly a possibility that these descending fibres participate in sympathetic or viscero-somatic reflexes, since several workers have shown that vasomotor and other responses follow visceral nerve stimulation (e.g. McDowall, 1935; McSwiney, 1938), and long ascending and descending pathways can participate in these reflexes (see Sato & Schmidt, 1973).

Reports concerning the descending pathway involved in micturition are conflicting and inconclusive. The location of the pathway would appear to be in the lateral funiculus, but its actual position has been ascribed variously to dorsal, ventral, intermediate and marginal layers (e.g. Foerster, 1936; Wang & Ranson, 1939*a*, *b*; Nathan & Smith, 1958; Kuru, Kurati & Koyama, 1959; Kerr & Alexander, 1964). Further, the conduction velocity of such a pathway is not conclusively established. If the pathway does not show convergence from hypogastric nerve afferents (see above), very few of the descending fibres in the present study would be eligible.

There have been several reports of descending fibres affecting transmission through ascending systems (e.g. Taub, 1964; Reynolds, 1969; Brown & Franz, 1969). It is quite possible that visceral ascending systems are subject to similar controls, as suggested earlier. The properties of any such paths remain unknown at present, but as it seems likely that they would receive some input from visceral afferents, it could be speculated that some of the descending fibres recorded here subserved such a function.

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