J. Physiol. (1982), **322**, *pp.* 35–43
With 4 text-figures
Printed in Great Britain

FACTORS THAT DETERMINE THE EXCITABILITY OF PARASYMPATHETIC REFLEXES TO THE CAT BLADDER

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(*Received 28 January 1981*)

SUMMARY

1. Spino-bulbo-spinal reflex responses could be recorded from the vesical branches of the pelvic nerve following electrical stimulation of afferents in the vesical or colonic branches of the pelvic nerves, the hypogastric or pudendal nerves. The latencies of responses from these different sources were similar.

2. Short latency responses could be recorded from the vesical branches of the pelvic nerve on electrical stimulation of descending pathways in the spinal cord and ipsilateral to and just below a hemitransection at the second cervical segment.

3. These responses were facilitated by increases in intravesical pressure, maximal facilitation occurring at about 30 mmHg. Conversely, increases in intracolonic pressure inhibited these parasympathetic evoked responses, the maximum effect being seen with intracolonic pressures of 40–50 mmHg.

4. These results suggest that the spino-bulbo-spinal responses that are recorded from the vesical parasympathetic efferents can be elicited from nerves innervating viscera other than the bladder, and also somatic structures. The effects of these spino-bulbo-spinal pathways on vesical parasympathetic efferents are dependent on antagonistic influences of intravesical and intracolonic pressure.

5. The pathways that mediate the changes in excitability in these reflexes appear to act at least in part at the termination of the bulbo-spinal limb of the reflex, and involve afferents in the pelvic nerves but not the hypogastric or lumbar colonic nerves.

6. It is proposed that the neurones that mediate this 'gating' action on the excitability of pelvic nerve reflexes are located in the sacral cord, and form a proportion of the population of interneurones described by McMahon & Morrison (1982*b*). In addition it is proposed that the neurones which mediate the ascending limb of the spino-bulbo-spinal reflexes are the long ascending neurones described by McMahon & Morrison (1982*a*).

INTRODUCTION

The two preceding papers have described a classification of neurones with vesical and/or colonic inputs in terms of their projections, the peripheral pathways of their inputs, their receptive fields and their transfer functions. This paper seeks to answer a number of questions concerning the functional roles of the sacral interneurones and the long ascending neurones in the micturition reflex.

It is believed that the neural basis of the micturition reflex involves a spino-bulbo-spinal pathway activated by vesical afferents in the pelvic nerve. On the hypothesis presented by de Groat (1975) any stimulus that activates the spino-bulbo-spinal pathway should cause micturition. This paper considers two main questions relating to this hypothesis. First, may units with long ascending axons that receive inputs from viscera other than the bladder contribute to the ascending limb of the micturition reflex? The results indicate that vesical parasympathetic reflexes may be elicited by stimulation of afferents from different viscera, and not solely by the pelvic nerve afferents from the bladder, as has been assumed by most previous workers. Secondly, if the spino-bulbo-spinal reflex can be elicited from non-vesical inputs, does a second, more specific pathway control the transfer of information from the descending pathways to the parasympathetic efferents?

These experiments suggest that the sacral cord contains a second more specific pathway that controls transmission between the bulbo-spinal axons and the vesical efferents. This second pathway is influenced by factors that determine micturition threshold, and is thought to be mediated by some of the sacral interneurones described in the previous paper. A hypothesis is presented that proposes the existence of a gating pathway in the sacral cord that modulates the activity of the spino-bulbo-spinal loop: the factors that control the activity of the gating and the gated pathways are discussed. The existence of a sacral pathway which controls some of the inputs to vesical parasympathetic neurones is a new finding that may provide a basis for the understanding of bladder reflexology following spinal transection.

METHODS

Experiments were performed on fourteen cats anaesthetized with alpha-chloralose (60 mg/kg, after initial induction with Fluothane). Supplementary doses of chloralose (10–30 mg) were given as necessary to maintain a constant depth of anaesthesia. The saphenous vein, one carotid artery and the trachea were cannulated. Arterial blood pressure was monitored and maintained above 100 mmHg. The animals were artificially ventilated with room air enriched to contain 40% oxygen. End-tidal CO_2 percentage was monitored and maintained between 3·5 and 4·5%. An oesophageal probe was used to monitor the animal's deep body temperature, which was maintained between 36 and 39 °C. The animals were paralysed with Flaxedil (20 mg).

The pelvic viscera were exposed via a long mid line laporotomy, and splitting and retraction of the pubic symphysis. The hypogastric nerves and lumbar sympathetic chain were crushed. Stimulating electrodes were placed around both hypogastric nerves (proximal to the point of crushing) and around one pelvic nerve. Stimulating electrodes were also placed around one pudendal nerve. The second pelvic nerve trunk was exposed, branches were cut near the bladder base, and recordings were made with silver electrodes from the proximal end. The recordings were amplified with an Isleworth pre-amplifier and averaged with a Neurolog averager. The bladder was cannulated via an incision made in its apex or via the urethra. The bladder pressure could be servo-controlled by applying air pressure to a reservoir connected to the urethral cannula in proportion to the differences between a reference signal and the signal from a pressure transducer that measured intravesical pressure. In this way intravesical pressure could be maintained at any desired level, independent of interventions that altered the contractile state of the bladder.

In most experiments the bowel was cannulated and stimulating electrodes placed around the colonic branches of the pelvic nerve. In some experiments a laminectomy was performed between the second and fifth cervical segments. After initial recordings had been made of reflex firing in pelvic preganglionic efferents, the cord was hemitransected at the second cervical segment, and a bipolar stimulating electrode, outside diameter 0·5 mm (Rhodes Instruments Ltd.) was placed in the cord, below the level of the section, at a site where low intensity stimulation (less than 100 μA,

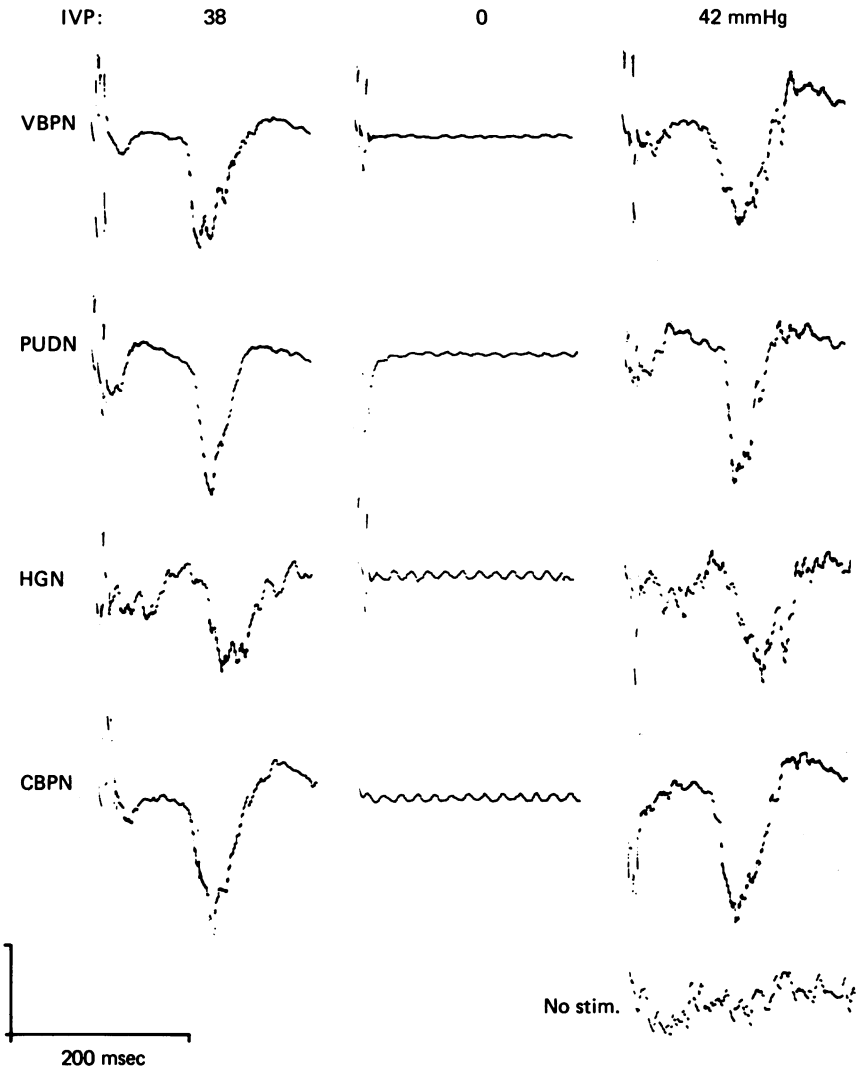

Fig. 1. The average of sixteen responses in vesical parasympathetic efferents following electrical stimulation (at start of sweep) of vesical branches of the pelvic nerve (VBPN, top row), pudendal nerve (PUDN, second row), hypogastric nerve (HGN, third row) and colonic branches of the pelvic nerve (CBPN, fourth row). An averaged control response using no electrical stimulation is shown on the bottom right. Responses were recorded at different intravesical pressures: 38 mmHg (first column) 0 mmHg (second column) and 42 mmHg (third column). Vertical calibration: 60 μV for responses in first row, and 30 μV for all others. All responses obtained from one animal.

400 msec square-wave pulses) at 20–30 Hz produced increases in bladder motility. In all experiments only one restricted area was found where such stimulation could produce large increases in bladder pressure. A diagrammatic summary of the innervation of the pelvic viscera is given in Floyd, McMahon & Morrison (1982).

RESULTS

Pelvic nerve discharges in the intact animal

Activity in pelvic nerve efferents was recorded, but when bladder pressure was less than 5 mmHg the nerve recording showed very little or no activity. Raising bladder pressure above approximately 20 mmHg evoked a noticeable efferent firing. Similarly,

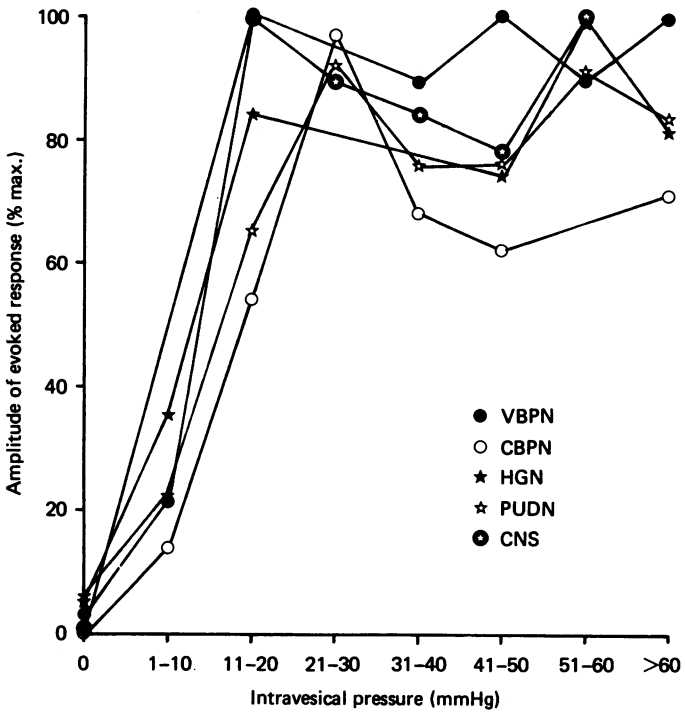

Fig. 2. The relationship between the amplitude of sixteen averaged evoked responses in the vesical parasympathetic efferents following electrical stimulation of the vesical (VBPN) or colonic (CBPN) branches of the pelvic nerve, the hypogastric nerve (HGN), the pudendal nerve (PUDN) or below a hemitransection of the spinal cord at the second cervical segment (CNS), at different intravesical pressures and low intracolonic pressure. The responses in all experiments have been pooled into bins of intravesical pressure as indicated on the abscissa.

stimulation of a pelvic nerve branch contralateral to the recording site produced no evoked responses when bladder pressure was near zero, but marked responses when bladder pressure raised (Fig. 1).

When the bladder was left in an isovolumic state with a volume greater than the micturition threshold, a series of spontaneous bladder contractions ensued. Stimulation of pelvic nerve afferents during this state was found to produce evoked potentials in pelvic nerve efferents during the contractions, but not during the periods of low pressure between the contractions. At high pressures, the latencies of evoked responses were found to be 87 $\pm$ 13 msec (mean $\pm$ S.E.). This pelvic-pelvic reflex has

been observed by several workers and is held to be the electrophysiological basis for the micturition reflex (Bradley & Teague, 1968; de Groat & Ryall, 1969).

Stimulation of hypogastric, pudendal and colonic branches of the pelvic nerves were all found to elicit evoked responses in vesical branch efferents of the pelvic nerve. The amplitudes of these responses were all dependent on intravesical pressure. Fig. 1 shows typical averaged mass waves recorded from the vesical branches of the pelvic nerve following stimulation of the vesical branches of the pelvic nerve, colonic

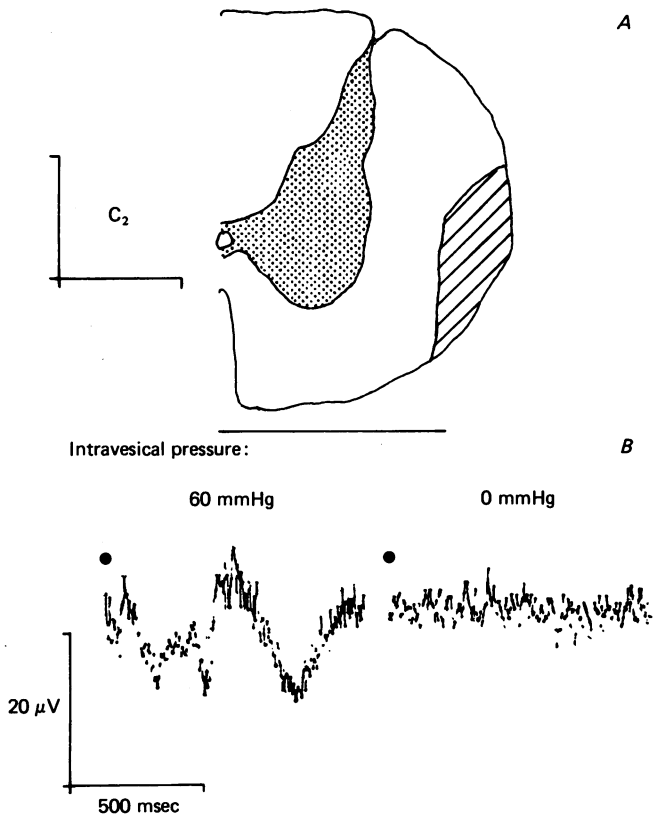

Fig. 3. *A*, the region in the second and third cervical segments from which electrical stimulation evoked bladder contractions and responses in vesical parasympathetic efferents. *B*, an example of averaged (16 sweeps) evoked responses (at high and low intravesical pressures) in the vesical branches of the pelvic nerve following electrical stimulation (100 μA, 400 μsec, pulses indicated by the dots) below and ipsilateral to a hemitransection of the spinal cord at the second cervical segment.

branches of the pelvic nerve, hypogastric nerve and pudendal nerve. The average latencies of evoked responses from different preparations in response to stimulation of hypogastric, colonic branches of the pelvic and pudendal nerve were 92 ± 15 msec, 80 ± 12 msec and 87 ± 6 msec (mean $\pm$S.D.) respectively. One feature commonly observed is shown in Fig. 1: the amplitude of the evoked wave was approximately twice as large when the vesical branches of the pelvic nerve afferents were stimulated as compared with the other afferent nerves.

The amplitude of the evoked responses showed a graded increase with increase in pressure. A plot of amplitude (as a percentage of maximum) against pressure is shown in Fig. 2, from pooled data. The variation from animal to animal was quite large possibly because of differing degrees of 'tone' in the bladder.

Responses after hemisection of the spinal cord

Bladder motility was transiently depressed following cord hemisection at the cervical level. The duration of the depression was usually about 10 min, during which period no micturition contractions could be elicited. When the bladder had contained just sufficient fluid to generate spontaneous bladder contractions prior to hemisection, it was usually found that an extra volume of fluid was required for the resumption of contractions; i.e. the micturition threshold had increased. However, the size of spontaneous micturition contractions was usually very similar before and after hemisection.

The effects of peripheral nerve stimulation were retested thirty minutes after hemisection, and responses in pelvic nerve efferents were essentially unchanged. The response still varied with bladder pressure, and the relationship between amplitude of the evoked potential and bladder pressure was unaffected.

A stimulating electrode was placed in the spinal cord, immediately below the lesion, at a site where low threshold stimulation (less than 100 μA, 400 μsec) produced increases in bladder motility. The frequency of stimulation needed to be greater than 20 Hz to induce changes in bladder pressure. This site was located in the ventro-lateral white matter of the spinal cord, as shown in Fig. 3*A*. Since the electrode was immediately below the level of the transection, it is probable that the responses were due to activation of a descending pathway. Single, or short trains of stimuli (4 in 10 msec), applied to the spinal cord at the active site were found to produce firing of pelvic nerve efferents. An early evoked wave was consistently seen; often a late wave was also present. The latency of the early wave was 39 ± 12 msec, whilst that of the late wave was 250 ± 65 msec (mean $\pm$ S.D.). The size of the early wave was usually about half the size of the pelvic nerve induced response, and the late wave was usually as big as, or bigger than, the early wave. Fig. 3*B* shows an example of the response evoked from the second cervical segment. The size of both evoked waves depended on bladder pressure, and the relationship between the amplitudes of the early wave and intravesical pressure is shown in Fig. 2 and is similar to the obtained response from stimulation of peripheral nerves.

Effects of varying colonic pressure

Activation of pelvic nerve afferents by raising intravesical pressure has been shown to facilitate the spino-bulbo-spinal responses evoked in efferents in the vesical branches of this nerve. In three experiments tests were performed to establish the relationship between amplitude of the responses and intracolonic pressure, in preparations in which the only afferent pathway from the colon was the colonic branches of the pelvic nerves. Intravesical pressure was raised to, and held at, a level such that the efferent evoked responses in vesical branches of the pelvic nerve were of a maximum amplitude, and the size of these responses was then determined at various levels of intracolonic pressure (Fig. 4). The Figure demonstrates that

increases in intracolonic pressure attenuate the size of these efferent evoked potentials in a graded fashion, and that responses evoked from different sources (including electrical stimulation of colonic branch afferents) were similarly affected. All evoked responses were abolished at intracolonic pressures in excess of 50 mmHg, and at 25–30 mmHg intracolonic pressure, responses were reduced to about 50 % of their control levels. When intravesical pressure was zero, raising intracolonic pressure did not facilitate spino-bulbo-spinal reflexes.

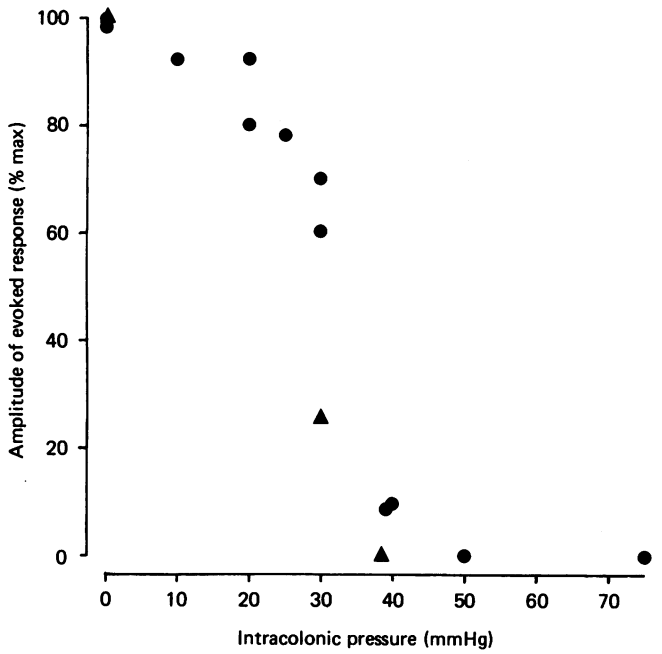

Fig. 4. The influence of intracolonic pressure on the amplitude of sixteen averaged evoked responses in the vesical parasympathetic efferents at constant high bladder pressure. Triangles indicate responses to stimulation of the colonic branches, and circles responses to stimulation of the vesical branches of the pelvic nerves.

Effects of repetitive stimulation of peripheral nerves

In two cats experiments were performed to determine whether repetitive stimulation of the vesical branches of the pelvic nerve could substitute for increases in intravesical pressure, in facilitating spino-bulbo-spinal responses. It was found that when the bladder was empty, stimulation of the hypogastric, pudendal or pelvic nerves could be made to induce an evoked wave in pelvic nerve efferents, at a similar latency to that occurring at high levels of intravesical pressure, providing that the stimuli followed a one second period of tetanization in the vesical branches of the pelvic nerve at 40 Hz. Such an effect was not seen when other nerves were repetitively stimulated.

Observations on animals with intact hypogastric nerves

When the pelvic nerves were cut, no evoked responses could be recorded following stimulation of pelvic nerve afferents, even though the intravesical pressure was high and the hypogastric afferent pathways were intact.

DISCUSSION

The results presented here modify the previously held view of the control over the vesical efferents in the pelvic nerve. Spino-bulbo-spinal reflexes may be elicited in these efferents by electrical stimulation of afferents of diverse origins, including the skin and muscle of the perineum, the colon and the bladder. Afferents running in sympathetic nerves from these viscera can also elicit these responses. Secondly, the excitability of these reflexes of diverse origin is dependent on intravesical pressure and on intracolonic pressure, and these influences have quantitatively similar effects on responses evoked from different sources. The intravesical pressure which resulted in 50% of maximal response was generally in the range 10–20 mmHg and the intracolonic pressure that caused 50% reduction in the evoked responses was in the range 25–30 mmHg. The nervous pathways that mediated these changes in reflex excitability were the branches of the pelvic nerve and not the vesical or colonic afferents of the sympathetic nerve trunks.

The hypothesis that has evolved as a result of these experiments is that intravesical pressure and intracolonic pressure have profound modulating influences on spino-bulbo-spinal reflexes that influence the vesical efferents of the pelvic nerve, and that these influences are powerful enough to gate, or control, the transmission between the descending bulbo-spinal pathway, and the pelvic nerve efferents. Supraspinal influences may therefore manifest themselves only if the intravesical and intracolonic pressures are appropriate, e.g. electrical stimulation of the colonic branches of the pelvic nerve can elicit a reflex response in vesical branches of the pelvic nerve at low colonic pressure, but fail to do so if colonic pressure is high. Some of the erratic nature of the bladder reflexes elicited by somatic nerve stimulation may also be explained by uncontrolled visceral pressures.

It seems likely that the gating or modulation of supraspinal influences occurs in the sacral cord: the early evoked responses from spinal cord stimulation with low currents are themselves dependent on the antagonistic influences of intravesical and intracolonic pressure.

One can conclude from this study that micturition is normally elicited by bladder distension, not because the spino-bulbo-spinal pathways carry information that is bladder-specific or only elicitable from the pelvic nerve, but because the activity of the non-specific pathways is modulated by a more specific gating mechanism that facilitates transmission in the final (and possibly other) sections of the pathway. This gating pathway makes use of the pelvic nerve afferents from the bladder and colon, and facilitates the spino-bulbo-spinal reflexes if bladder pressure is high and colonic pressure is low. It has been shown that the hypogastric nerves cannot substitute for the pelvic nerves in the gating mechanism.

This proposed scheme of the organisation of pathways controlling pelvic nerve efferent activity has support from the observations of McMahon & Morrison (1982*a*, *b*). They have described a group of sacral interneurones (group A) whose mechanical thresholds, patterns of convergence and response to intravesical and intracolonic pressure changes correspond with those of the gating pathway seen here. In addition, there was a close correspondence between the mean intravesical and intracolonic pressure thresholds of group A interneurones and the pressures in the

bladder and colon that produced 50% of maximal facilitation or inhibition, respectively.

Further, the finding that spino-bulbo-spinal reflexes could be evoked from several peripheral sites is in accordance with the observations of McMahon & Morrison (1982*a*) that most neurones with long ascending projections and concerned with transmitting information from bladder to brain can be activated from all of the peripheral nerves tested in this study.

Colonic pressure has an important influence, that can over-ride the facilitatory effects of intravesical pressure. It may be that other influences, e.g. somatic inputs, can also modulate the gating mechanism. McMahon & Morrison (1982*b*) found that the group A interneurones had pudendal nerve inputs and some had hind limb receptive fields in addition. These properties may be responsible for some of the somato-vesical reflexes described by McPherson (1966), Sato, Sato & Schmidt (1979), Kock & Pompeius (1963) and Sundin, Carlsson & Kock (1974). The dual nature of the pathways in the micturition reflex, and the convergent inputs on to them, modify the previously existing view of micturition and allow the inclusion of demonstrable somatic and visceral influences on pathways which previously have been regarded as only transmitting information of vesical origin.

The possibility that a sacral cord gating mechanism could remain in operation when the distal segments of cord became hyperexcitable after spinal shock could be the basis of automatic micturition: weak somatic inputs that in intact animals contribute little to the gated pathway may dominate after removal of the bulbo-spinal influences. Such a mechanism would not require gross changes in synaptic connexions.

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