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# A possible new reflex pathway for micturition after spinal cord injury

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In order to restore bladder function after spinal cord injury, a controllable new reflex pathway has been established in rats. It involves a somatic reflex arc with an artificially modified efferent branch which passes the somatic motor impulses to the bladder. This is achieved by intradural microanastomosis of the left L4 ventral root to L6 ventral root, while leaving the L4 dorsal root intact as a starter of micturition. The 'skin–CNS–bladder' reflex pathway is designed to initiate voiding by scratching the skin. After axonal regeneration, 15 of the 24 rats with the new pathway underwent electrophysiological study. Single stimuli (0.3–3 mA, 0.02–0.2 ms duration) to the left L4 nerve resulted in evoked potentials (0.5–1 mV) recorded from the left L6 nerve distal to the anastomosis. The bladder detrusor contraction was very quickly initiated by trains of the stimuli and bladder pressures increased rapidly to levels similar to controls. Neural tracing study with horseradish peroxidase (HRP) on six rats with the pathway demonstrated that the somatic motor axons regenerated successfully into the pelvic nerve, and the bladder was reinnervated by the L4 somatic motor neurons. The bladder contraction can also be initiated by electrostimulation of left sciatic nerve as well as scratching the L4 related skin. A new concept may be derived from the skin–CNS–bladder reflex pathway: the impulses delivered from the efferent neurons of a somatic reflex arc can be transferred to initiate responses of an autonomic effector.

*Keywords*: neuropathic bladder; reinnervation; spinal cord injury.

## Introduction

Neuropathic bladder dysfunction caused by spinal cord injury presents a major health problem. In the United States there are more than half a million patients who need to regain bladder control after spinal cord injury, and an additional 50,000 persons will sustain a spinal cord injury each year.[1] Finding a way to solve this problem would not only reduce the morbidity rate in these patients, but would also improve their quality of life.

Research into the rehabilitation of the neuropathic bladder has focused on producing voiding by electrical stimulation. After the failure of initial attempts to induce micturition via electrical stimulation of the spinal cord,[2] and the disappointing results of stimulating the bladder muscle or pelvic nerve directly,[3,4,5] significant progress was achieved through stimulation of sacral motor nerves (ventral roots).[1,6] This procedure, however, has not become an accepted treatment of choice for the majority of patients with spinal cord injury, because the results are far from universal.[7] In addition, two complications can present: (1) failure of the electrode, which could be very difficult to reimplant, and (2) mechanical or electrical damage of the nerve which may be permanent.

An alternative approach has been to investigate restoring innervation to the lower urinary tract after spinal cord injury. Over the years, less than 20 papers have been published investigating bladder reinnervation.[8] It was reported that anastomosis of a lumbar spinal nerve (L7) to a sacral spinal nerve (S1) in cats resulted in significant bladder reinnervation. Stimulation proximal to the anastomosis can induce bladder muscle contraction. However, this

response was only 56% compared to controls.[9] Though the data show that axonal regeneration can occur after the crossover anastomosis, many questions remain unanswered. First, this technique uses a mixed motor and sensory nerve permitting possible inappropriate connections. For example, a motor axon in the proximal branch may grow into an endoneurial tube in the distal sensory branch, resulting in a nonfunctional regeneration. Second, since electrical stimulation of the ventral root (theoretically more effective than reflex stimulation) can initiate only 56% of a normal detrusor contraction, natural stimulation, i.e. with a full bladder, may be too weak to initiate normal micturition.

Therefore, in order to regain bladder control in a more natural and effective way, it is critical to establish a pathway which can effectively control both motor and sensory aspects of micturition. A new approach has been investigated to establish a skin–CNS–bladder pathway for restoring controllable reflex micturition.

## Materials and methods

The establishment of the skin–CNS–bladder reflex pathway is based on the assumption that the efferent root of a somatic reflex arc above the spinal micturition center may be able to regenerate into the autonomic efferent root which controls the bladder, thus the bladder function may be controlled via the somatic arc (Fig 1).

Male Sprague-Dawley rats were divided into three groups of 15, 6, and 3 respectively. The surgical anastomosis was performed on all groups. Group I was used in neurophysiological experiments. Group II was used in horseradish peroxidase (HRP) neural tracing studies, and group III was for long term observation and function test of the skin–CNS–bladder reflex pathway. An additional 10 rats were used for normal control studies.

### *Surgical anastomosis*

The rats were anesthetized with 50 mg/kg pentobarbital plus 0.05 mg atropine intraperitoneally, supplemented with 2% halothane and oxygen at a rate of 1–2 l/min via a free mask. Under sterile conditions a lateral hemilaminectomy was performed on the left side exposing the dorsal root (DR) ganglia of L4 through S1. Ventral roots (VR) of L4 and L6 were transected and the proximal end of the L4 VR anastomosed to the distal end of the L6 VR (Fig 1). In order to ensure that the bladder contraction is only initiated through the modified left L4 somatic reflex arc, about 3 cm proximal L6 VR was removed and special caution was taken to keep the L4 DR, the proximal L4 VR and the distal L6 VR intact. The anastomosis

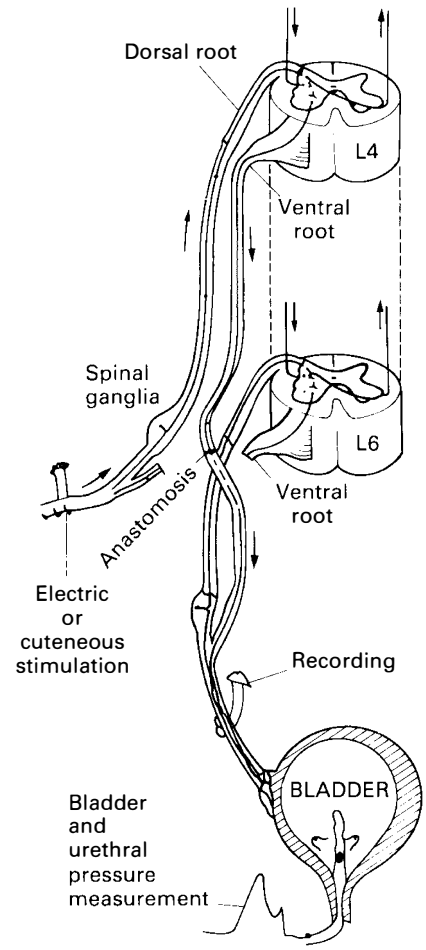


**Figure 1** Illustration of the skin–CNS–bladder reflex pathway in rat. The right side serves as control.

was covered with a tube made of subcutaneous fascia. Other roots were intact. The wound was closed and the rats allowed to recover and survive for 3 months. This should allow adequate time for axon regrowth at a rate of 1–2 mm/day over a length of approximately 45 mm.[10]

*Neurophysiological experiments*

Following a 3 month recovery period, group I rats were anesthetized with halothane and oxygen, a venous catheter placed in the tail vein, and the anesthetic changed to Chloralose, 50–100 mg/kg IV. A tracheotomy was performed for artificial ventilation after relaxant (Flaxedil) was applied. Through a midline abdominal incision, a bladder catheter was introduced through the posterior urethra and connected to a pressure transducer to record bladder pressures. The ureters were ligated, divided, and allowed to drain into the abdomen. The L4 and L6 nerves on both the operated and the unoperated sides were dissected extradurally (to avoid damaging the nerves in the scar tissue) and studied, the unoperated right side serving as a control. As shown in Figure 1 an electrical stimulus was applied to the L4 nerve and an electrical response recorded in the L6 nerve and a bladder contraction recorded via the bladder catheter. The electrical stimulus was a monophasic square wave pulse between 0.2 to 3 mA, of varying frequency and duration, generated by a Grass S88 stimulator, delivered through a Grass SIU5 stimulus isolation unit and silver bipolar wire hook electrodes. The electrodes were placed around the left L4 nerve 1 cm distal to the L4 spinal ganglia. The left L4 nerve had only sensory axons left and served as a pure afferent in the skin–CNS–bladder reflex pathway. The evoked potentials were recorded through bipolar silver wire hook electrodes through a Grass P511 amplifier with high impedance probe. The recording electrodes were placed around the left L6 nerve, 1 cm distal to the L6 spinal ganglia. These signals were monitored on a Tektronix 2211 storage oscilloscope and stored in a 80-286 personal computer via an interface. Bladder pressure was also monitored on the oscilloscope and recorded on a Western Graphtek 3700 polygraph from the output of a Hewlett-Packard pressure transducer. The rest bladder gross pressure was adjusted to around 15 cm$H_2O$.

*HRP tracing*

Approximately 3 months after the anastomosis, group II rats were anesthetized with pentobarbital, 50 mg/kg IP. The 25% solution of HRP in 2% dimethylsulfoxide was freshly prepared. The left L6 nerve distal to the anastomosis was dissected and 20 $\mu$l HRP was microinjected into the nerve to label retrogradely motor neurons regenerating through the anastomosis in four rats. In another two rats, HRP was applied to the pelvic ganglia. The wound was closed and the rat allowed to recover for 48 hours. Then the rat was reanesthetized for perfusion and fixation with normal saline and 1% paraformaldehyde plus 1% glutaraldehyde solution. A laminectomy of T10–S3 was performed and spinal cord removed with caution. The segments of T12, L1, L2, L3, L4, L5, L6, S1 and S2 were separated and sectioned for standard tetramethylbenzidine tissue processing and dark field light microscopic examination.[11]

Control HRP tracing studies included applying HRP to right side L6 nerve ($n = 2$), or to pelvic ganglia ($n = 2$) in normal rats. The location, number and type of the neurons labelled were compared with those of the group II animals.

*Long term observation and final function test*

Group III rats were allow to survive at least 1 year after surgical microanastomosis for long term observation, which included effect of left L6 VR transection on urination, effect of Left L4 transection on left rear limb function, as well as the long term result of the skin–CNS–bladder reflex pathway. For the final function test, the animal was anesthetized, a catheter was inserted to the bladder for bladder pressure measurement via a transducer connected to the polygraph. Both side distal sciatic nerves and L4 to S1 nerves were dissected. In addition to the

electrophysiological studies in group I, electrostimulation on sciatic nerves and scratching on the L4 related skin zone were performed to initiate bladder contraction. Afterwards, a laminectomy was done from T8 to S2 and the spinal cord was transected horizontally at the L5 segment level. The control side (right) L4–S2 as well as the left side L5, S1 and S2 were transected, to ensure the bladder was only controlled through the skin–CNS–bladder reflex pathway. Then, stimulation on left L4, on sciatic nerves, and scratching the skin were performed again for comparison of evoked bladder contraction.

## Results

After a minimum of 3 months regeneration, the new pathway was studied electrophysiologically or by HRP neural tracing. The results revealed both histological and physiological evidence of a highly functional new reflex pathway.

*Electrophysiology*

Of the 15 animals in group I, 13 were studied electrophysiologically. (One rat died due to anesthesia, and one secondary to excessive heat exposure). One failed due to technical problem with the anastomosis. In the remaining 12 animals, very similar results were obtained. Single stimuli (0.3–3 mA, 0.02–0.2 ms duration) to the left L4 nerve resulted in evoked potentials (0.5–1 mV) recorded from the left L6 nerve distal to the anastomosis (Fig 2). No potential could be recorded on the contralateral L6 nerve when the L4 nerve of either side was stimulated. The bladder detrusor contraction was very quickly initiated by trains of the stimuli at the rate of 5–20 per second, and bladder pressures increased rapidly to levels similar to controls (Figs 3, 4). The bladder pressure could increase to as high as 45 $cmH_2O$, with an average of $38 \pm 7$ $cmH_2O$.

*HRP neural tracing*

HRP retrograde tracing study on two normal rats and six rat models with the new reflex pathway provided histological and

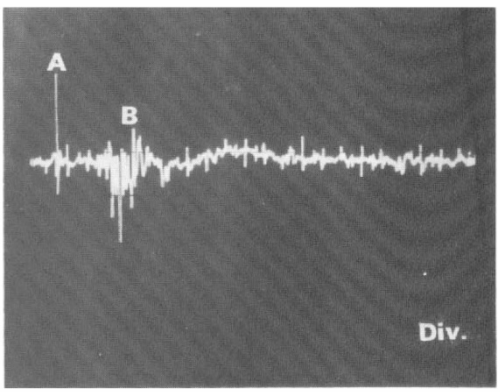


**Figure 2** After at least 3 months regeneration, single stimuli applied to the left L4 spinal nerve (0.6 mA, 0.2 ms duration, threshold 0.4 mA/0.02 ms) produced an evoked potential recorded from the left L6 spinal nerve (5 ms/div, 0.5 mV/div). The potential had a latency of 7 ms, a duration of 4 ms, and an average voltage of 0.6 mV. A: stimuli's artifact; B: evoked potential.

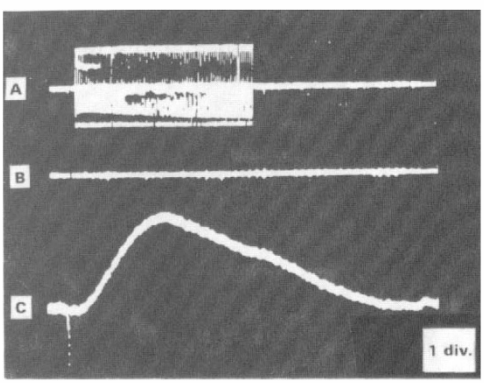


**Figure 3** Trains of stimuli (1.2 mA, 5 Hz, 0.2 ms duration) to the left L4 nerve resulted in rapid bladder pressure rise similar to the control. A: potentials recorded on the left L6 nerve; B: no potential could be recorded on the right L6 nerve (control); C: bladder pressure (10 $cmH_2O$/div, 2 s/div).

morphological bases for the skin–CNS–bladder reflex pathway. In the normal rat, spinal micturition center is located in L6 and S1 segments. The parasympathetic preganglionic neurons in the lateral band of L6 and S1 were labelled when HRP was applied to the pelvic ganglia (Fig 5). In rats with the

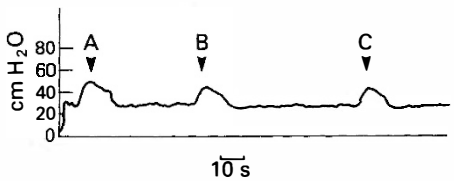


**Figure 4** Comparisons of maximum bladder contraction initiated by trains of stimuli on A: the right L6 nerve (control), 0.6 mA, 5 Hz, 0.05 ms duration; B: the left L6 nerve, 0.6 mA, 5 Hz, 0.05 ms; and C: the left L4 nerve, 1.2 mA, 5 Hz, 0.2 ms duration.

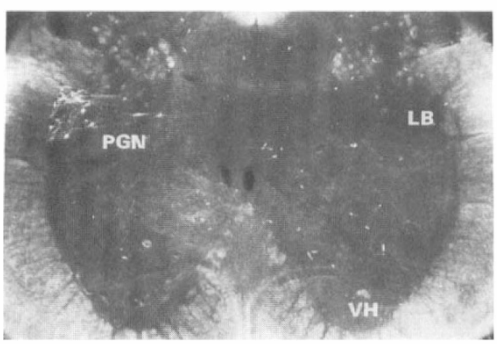


**Figure 5** Neural tracing study by applying HRP to the pelvic ganglion show that the normal bladder was innervated by preganglionic neurons located in the lateral band of the L6 and S1 segments in the rat. PGN = preganglionic neurons; VH = ventral horn; LB = lateral bond.

skin–CNS–bladder pathway, somatic motor neurons in the L4 ventral horn were labelled when HRP was applied to the left L6 nerve distal to the anastomosis (four rats) as well as to the left pelvic ganglia (two rats) (Fig 6). Since HRP can be picked up and transported to neurons by living axons only, these results demonstrate histologically the functional regeneration of somatic motor axons into an autonomic efferent root.

*Further functional tests of the skin–CNS–bladder pathway*

Since the final goal of the project is to apply the skin–CNS–bladder pathway procedure to the patients who need to regain bladder control, and to allow them to initiate voiding themselves by scratching the related skin, further functional evaluation of the pathway was carried out on three long term survival animal models.

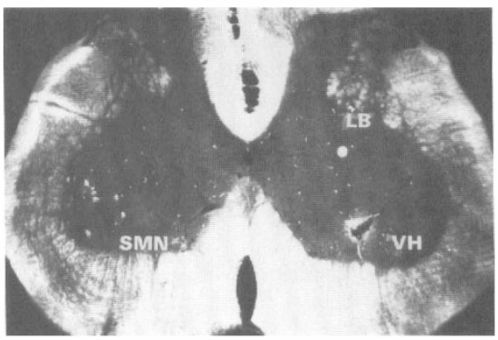


**Figure 6** In the rats with the new pathway, somatic motor neurons in left L4 ventral horn were labelled when HRP was applied to Left L6 spinal nerve (four rats) as well as pelvic ganglion (two rats). SMN = somatic motor neurons; VH = ventral horn; LB = lateral bond.

These rats had survived at least 1 year after the initial microanastomosis of the ventral roots. As the sciatic nerves of rats consist of neural fibers from both L4 and L5, a small incision was performed on both legs to exposure the distal sciatic nerves. When electrostimulation with the same parameters as used in group I electrophysiological studies was applied to the left sciatic nerve, a bladder contraction was initiated and bladder pressure increased, though less rapidly, to the level similar to those caused by the L4 stimulation or the direct L6 stimulation as shown in Figures 3 and 4. Similar bladder response was also initiated by scratching the left L4 innervated skin zone (Figs 6, 7, 8). Electrostimulation on contralateral L4 nerve, or sciatic nerve or scratching the right side leg skin was not able to initiate bladder contraction.

Bladder responses were not changed after the spinal cord horizontal transection just below L4 and the spinal nerve transection of the right L4 to S2, the left L5, S1 and S2, which ensured the bladder was only controlled via the skin–CNS–bladder reflex. Electrostimulation with the same parameters on left L4 or sciatic nerve, as well as

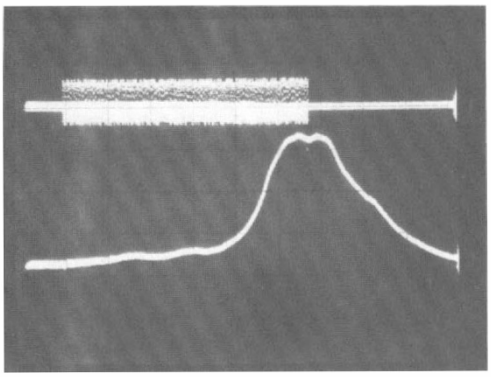

**Figure 7** Bladder contraction was initiated by electric stimulation (1.2 mA, 0.2 ms duration, 5–20 Hz) on distal left sciatic nerve. Upper: record on proximal left sciatic nerve, maximum evoked potential = 0.8 mV. (2 s/div, 1 mV/div); Lower: bladder pressure increased from 10 to 40 $cmH_2O$ (2 s/div, 10 $cmH_2O$/div).

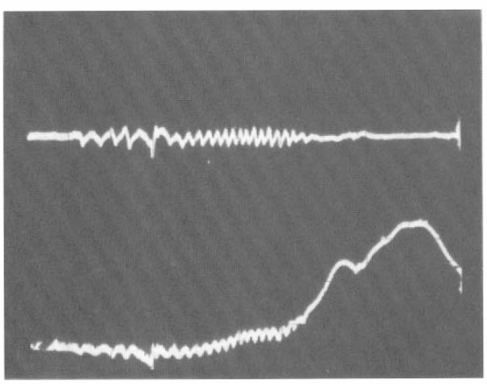

**Figure 8** Bladder contraction was initiated by scratching the skin of left leg. Upper: record on proximal left sciatic nerve to show scratching period; Lower: bladder pressure increased from 10 to 40 $cmH_2O$ (2 s/div, 10 $cmH_2O$/div).

scratching skin, can cause similar types of bladder contraction.

## Discussion

A neuropathic bladder caused by spinal cord injury presents a major social and medical problem. The condition is associated with poor quality of life because of the uncontrollable bladder, and significant morbidity and mortality due to recurrent urinary infections and renal failure. Various investigations aimed at a better understanding and treatment of the problem have been carried out for nearly a century and intensified in recent decades. Though important achievements have been made, no satisfactory treatment has been established to date. The only clinically applicable method to produce voiding has been sacral roots electrostimulation; however, the results with this procedure are inconsistent. In addition, mechanical malfunction of the stimulating devices requires difficult surgical replacement, and long term stimulation may result in deleterious effects to the nerves, and the dorsal rhizotomy may sacrifice the residual sensory function which is important to the patients.

There are very few papers in the literature discussing the possibility and mechanisms of cross-reinnervation with foreign nerves. Most of them concern the fate of mammalian sympathetic ganglion cells (superior cervical ganglion) reinnervated by vagus nerve[12–16] or by the fifth cervical nerve.[13,17] These studies demonstrated to some extent that foreign nerves could regenerate into the sympathetic ganglia and form synapses, and that the new connection was capable of being activated by electrical stimulation.[12–18] However, the consequences of activation of the new connections by normal nerve impulse traffic were not investigated. It was not reported or proved histologically and functionally that the pelvic ganglia could be reinnervated via somatic fibers, by which the bladder function could be restored.

This investigation demonstrated that the somatic motor root above the spinal micturition center can be used to reinnervate the bladder through axonal regeneration, thus establishing a new skin–CNS–bladder reflex pathway. This new pathway consists of a somatic reflex arc with a modified efferent branch, which sends the somatic motor impulses to the autonomic effector. This study on the rat models showed that strong bladder contraction could be initiated by activating such a new reflex pathway. We believe that anastomosing a motor root above the lesion to the sacral root is most desirable in overcoming the possible

problem of unpredictable time-course and diminishing that of irradiation. This, however, may not be technically practical in the higher spinal lesion cases, because the higher the spinal segment, the shorter the roots within the spinal canal. To assure a no tension microanastomosis of the roots, a shorter regeneration period and a better result, we would suggest that, no matter where the spinal lesion could be, the nearest lumbar efferent root be chosen as the proximal stump, provided that the related lumbar spinal segment and afferent root are intact. In fact, the thresholds of paralyzed body surface to the various sensory inputs may become much higher, and some common stimuli may not be able to result in any response as they may in normal body.

This investigation also provided histological evidences that somatic motor axons successfully and functionally regenerated into the efferent branch of an autonomic nerve. Considering the contrary interpretations and uncertainty which are always around the electrophysiology, this is very important and convincing. HRP neural tracing has been proved to be a very reliable technique in neurological research. HRP can only be picked up by living axons and transported retrograde or antegrade inside the axons. It is, however, the first report of successful HRP application in such a special retrograde tracing from pelvic ganglia to the lumbar somatic motor neurons via the regenerated axons. The neural tracing technique, combined with electron microscopic study, will be able to demonstrate the ending point of the regenerating axons. This is very important since where the axons terminate will affect how the skin–CNS–bladder pathway works and how the two different neural systems (somatic and autonomic) cooperate in the 'cross-wired' reflex pathway. Theoretically, the majority of L4 motor axons, which regenerate into the L6 autonomic endoneurial tubes, should terminate at the pelvic ganglia and synapse with postganglionic axons. Except in cases of extensive pelvic and bladder trauma, the postganglionic neurons and postganglionic axons are generally intact in spinal cord injury cases. This constitutes an important part of the skin–CNS–bladder reflex pathway. The neural tracing with HRP in this study was originally designed for retrograde labelling only, in order to determine whether axonal regeneration through the anastomosis had occurred. Further study with antegrade neural tracing is planned to identify where regenerating axons terminate.

Detrusor-external urethral sphincter dissynergia can further complicate the clinical presentation of neuropathic bladder. Different strategies have been proposed to deal with the phenomenon, most of them aimed at eliminating the role of external urethral sphincter. These include transection of pudendal nerves, transurethral resection of the external sphincter, and urinary diversions and are clearly not physiological solutions. Ideally, both bladder and external sphincter functions should be restored to cooperate in micturition. The skin–CNS–bladder pathway procedure may provide such result. Since the L6 spinal nerve in rat (as S2, S3, and S4 in the human) consists of both autonomic fibers, which form the pelvic nerve, and somatic fibers which form the pudendal nerve, the simultaneous reinnervation of both bladder and external urethral sphincter should be the logical result should the L4 motor axons regenerate through. This, however, needs to be confirmed in further studies on larger animals which would include urodynamic evaluations and external urethral sphincter EMG recordings. Because high frequency rhythmic contractions of external sphincter normally occur during voiding in the rat, the rat may not represent a good model for examining the detrusor-external sphincter dissynergia.

The skin–CNS–bladder reflex pathway for micturition seems to be advantageous over other options available in treatment of neuropathic bladder caused by spinal cord injury. It requires relatively minor surgery. It does not involve implantations of electrodes or other mechanical device, though a portable skin stimulator may be helpful in cases where the scratching or squeezing is not strong enough to evoke a steady stream of motor impulses to the bladder. It provides unique voluntary control of bladder emptying. Moreover, many spinal cord

juries are incomplete and, therefore, some sensory functions may remain or recover afterwards. As this procedure does not require dorsal rhizotomy, this residual sensory function is preserved, which is important for the patient's sexual function and awareness of noxious stimuli.

The skin–CNS–bladder reflex pathway is practical in the rat model and may have a potential of clinical application. Theoretically, a new concept may be derived: the impulses delivered from the efferent neurons of a somatic reflex arc can be transferred to induce response of an autonomic effector.

## Acknowledgements

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## References

1 Tanagho EA, Schmidt RA (1988) Electrical stimulation in the clinical management of the neurogenic bladder. *J Urol* **140**: 1331–1339.
2 Jonas U, Tanagho EA (1975) Studies on the feasibility of urinary bladder evacuation by direct spinal cord stimulation. *Invest Urol* **13**: 142–150.
3 XIE Tong (1965) Electrical stimulation on bladder detrusor via an implanted electrode. *Urol* **2**: 82–86. (Letter, Chinese).
4 Hold T, Agrawal G, Kantrowitz A (1966) Studies in stimulation of the bladder and its motor nerves. *Surgery* **60**: 848–853.
5 Holmquist B (1968) Electromicturition by pelvic nerve stimulation in dogs. *Scand J Urol Nephrol* (Suppl), **2**: 1.
6 Brindley GS, Polkey CE, Rushton DN *et al* (1986) Sacral anterior root stimulator for bladder control in paraplegia: the first 50 cases. *J Neurol Neurosurg Psychiatry* **49**: 1004–1011.
7 Bonder DR (1990) How electrical stimulation improves micturition. *Contemp Urol* **3**: 39–43.
8 Vorstam B, Schlossberg SM, Kass L (1987) Investigation on urinary bladder reinnervation: historical perspective and review. *Urology* **30**: 89–96.
9 Vorstam B, Schlossberg SM, Kass L, Devince Jr CJ (1986) Urinary bladder reinnervation. *J Urol* **136**: 964–969.
10 Seil FJ (1983) *Nerve, Organ, and Tissue Regeneration: Research Perspectives*. Academic Press, New York: 21.
11 Morgan C, deGroat WC, Nadelhaft I (1986) The spinal distribution of sympathetic preganglionic and visceral primary afferent neurons which send axons into the hypogastric nerves of the cat. *J Comp Neurol* **243**: 23–40.
12 Langley JN (1898) On the union of the cranial autonomic (visceral) fibres with the nerve cells of the superior cervical ganglia. *J Physiol* (London) **23**: 240–270.
13 Langley JN, Anderson HK (1904b) The union of different kinds of nerve fibers. *J Physiol* (London) **31**: 365–391.
14 Guth L (1956) Functional recovery following vagosympathetic anastomosis in the cat. *Am J Physiol* **185**: 205–208.
15 Ceccareli B, Clementi F, Mantegazza P (1971) Synaptic transmission in the superior cervical ganglion of the cat after reinnervation by vagus fibers. *J Physiol* (London) **216**: 87–98.
16 Purves D (1976) Competitive and non-competitive reinnervation of mammalian sympathetic neurons by native and foreign fibers. *J Physiol* (London) **261**: 453–475.
17 McLachlan (1974) The formation of synapses in mammalian sympathetic ganglia reinnervated with preganglionic or somatic nerves. *J Physiol* (London) **237**: 217–242.
18 Osterberg, AJC *et al* (1976) A quantitative comparison of the formation of synapses in the rat superior cervical sympathetic ganglion by its own and foreign nerve fibers. *Brain Res* **107**: 445–470.