Artificial Control of the Lower Urinary Tract by Sacral Root Stimulation

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This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Glasgow.

March 1995

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To my mother, Emilia, whom I shall never forget.
The great absence during these three years.

To my husband Teofilo, who supports and
encourages me to persevere in my dreams.
And to my daughter Carolina for coping with the
long hours taken from our family life.

Acknowledgement

This thesis is the fruit of a strong desire to find further improvement in the treatment of my wee patients in Brasil. However, my personal efforts alone would not have been enough to develop the work which I present today. There are a number of people whom I would like to thank.

This thesis would not come to reality without the support of Dr Ronald Hugh Baxendale, my supervisor during these years of PhD studies at the University of Glasgow. I would like to thank you, Ron, for all the clear suggestions to improve my work. Also, for the fruitful discussion during our meetings, I learned a lot from them. I will especially remember your care and patience when I was building up my knowledge in electrophysiology. And your supportive attitude and understanding during the harder times.

Dr Margaret Gladden, Dr David Pollock and Mr Peter Edmond for their suggestions which greatly contribute to improve the presentation of this work.

Prof. Carlos D'Ancona, my former supervisor in Brasil, for all the motivation and encouragement at the beginning of the PhD, and for his advice in Urodynamic Evaluation.

Mrs Marion Kusel for the technical support during the experiments. Marion became a good friend, who encouraged me towards the PhD.

Dr Dennis Fitzpatrick for his collaboration during the experiments and for the use of the cuff electrode and the stimulator during this work.

Mr David McLaren, and the Robertson Centre of Biostatistics, University of Glasgow, for the statistical advice.

Dr John Lockhart for reviewing the chapters and Dr Bill Ferrell for his support and interest in the project.

To CNPq and MRC for the financial support towards this research. Also to Fundação Oswaldo Cruz who granted me research leave during the time that I studied in Glasgow.

Dr Ian Montgomery, for his help and advice with the histological sections presented.

There are other persons who did not help directly with the research, but were equally important for the final result, and to whom I am also grateful. They helped me in the difficult times during this Ph.D., and I shall always remember them.

I will be always in debt to Mrs Alison Spurway. Alison was responsible for my permanence as a student in the University, and without her support I should never have finished this thesis.

Mrs Heather Collins for her friendly help in finding my way in the Lab in the earlier experiments.

To all the staff and colleagues from the department of Physiology for providing such a nice and friendly environment. You make Scotland my second home.

To Dr Antonio Roberto Richa Nogueira, Paulo Roberto Boechat and all the staff at the Paediatric Surgery department in Brasil for their constant motivation.

Finally, I would like to thank my family who always encourages my work. You are always present in my life.

Preface

The experiments described in this thesis were performed by the author in Dr Ronald Baxendale's laboratory within the Institute of Physiology, University of Glasgow, between January 1992 and March 1995.

Preliminary reports of the results have been published. These are related in "Publications and Presentations".

Finally, this thesis was composed by the author, in accordance with the regulations of the Faculty of Medicine, The University of Glasgow.

Abstract

Micturition disorders are humiliating disabilities which cause major clinical, social and financial problem for a substantial part of the population. In many cases it lacks any adequate conventional solution. The clinical application of the anterior sacral root stimulation introduced by Brindley and his co-workers (1992) has been one of the most successful application of functional electrical stimulation to control the lower urinary tract. It has been used in several countries world-wide to control micturition. However, its more widespread acceptance is limited by the unsuitable recruitment characteristics of bipolar stimulating electrodes. Normal micturition requires co-ordinated bladder contraction and sphincter relaxation. The sacral roots contain large somatic motor fibres supplying the skeletal muscle of the external sphincter mixed in with small autonomic motor fibres supplying the bladder. During sacral root stimulation the larger fibres are activated more easily than the smaller ones and so, the external urethral sphincter contracts and close before the bladder is activated, making normal voiding difficult. The solution would be reverse that recruitment selection and allows bladder contraction with a relaxed sphincter.

This research tests a tripolar stimulation strategy which is thought to be able to activate selectively small autonomic motors axons whilst blocking excitation in larger ones. This approach is based on initial mathematical modelling studies (Fitzpatrick, 1991). The construction of a tripolar electrode and the choice of stimulation current intensities and ratios were guided by the model predictions.

The simulation results of the modelled tripolar electrode predict that the generation and propagation of action potentials in the larger axons can be anodally blocked at one end of the cuff electrode, called the

blocking anode. Also, a second prediction is that is possible to regulate the intensity of the anodal current at the other side of the cuff electrode, called escape anode, in order to provide the selective blocking of the larger axons whilst allowing for the escape of action potentials in the smaller fibres.

Two series of experiments were carried out to test the model predictions.

The first series of experiments were developed to test the electrode and stimulation parameters, and if it is possible to achieve an anodal block at the blocking anode. Experiments performed in isolated rabbit sciatic nerve trunks, showed that the propagation of action potentials in all axons can be blocked at one end of the cuff, while unidirectional propagation of action potential still could be achieved at the escape end with tripolar stimulation. Generally, more current was needed to produce a block during the experiments than expected from the model, which can be explained because physiological circumstances during the experiments were not calculated. Block was achieved more frequently at an anodal ratio of 9:1 and trains of pulse of 0.3msec duration, confirming the model predictions.

The second series of experiments were developed to test if it is possible to regulate the intensity of the anodal current at the escape anode, in order to provide the selective blocking of the larger axons whilst allowing for the escape of action potentials in the smaller fibres. Unilateral ventral sacral root stimulation was performed on adult males New Zealand white rabbits which were deeply anaesthetised with a mixture of 1-3% halothane in nitrous oxide and oxygen. Trains of pulse of 0.3msec duration lasting up to 2sec at 20Hz were delivered. Simultaneous recording of bladder and urethral pressures were made by conventional urodynamic methods. The root which produced the

stronger bladder/urethral sphincter response was used throughout the experiment. The responses to stimulation of bladder, external urethral sphincter and coccygeal (skeletal) muscle, which is activated by stimulation of the same root, were compared during bipolar and tripolar stimulation.

No spontaneous activity was recorded in the bladder or urethral sphincter when the animal was deeply anaesthetised. It was possible to produce activity in the lower urinary tract in 27 experiments. Average bladder pressures of 30cm H_2O was developed during stimulation.

Using bipolar stimulation, the lowest threshold currents were always associated with skeletomotor axons supplying muscles in the hip and in the base of the tail. Further increase in stimulus intensity recruited axons supplying the external urethral sphincter and the highest thresholds were associated with the parasympathetic axons supplying the bladder. These results confirmed the expected recruitment characteristics during conventional stimulation and were highly significant ($p<0.0001$).

Using tripolar stimulation it was possible to reverse the bipolar recruitment sequence. The bladder was activated at lower thresholds than with bipolar stimulation. More significantly, bladder contractions were achieved without concurrent sphincter activity. Also, bladder pressure was higher than the sphincter pressure and it was stronger enough to trigger voiding. This reversal of the recruitment was statistically significant ($p<0.001$)

The results reported here confirm that selective control of the lower urinary tract by tripolar electrodes on the sacral anterior root is possible. The predictions of Fitzpatrick's modelling studies have been largely confirmed. These results are in agreement with recent reports of an essentially similar project carried out in dogs (Rijkhoff et al, 1994).

Although the model predictions were confirmed, the design of the tripolar cuff electrode used during this project would not allow its use in chronic experiments or in patients, since it requires to split the nerve in the proximal end.

The author believes this technique has potential application in neurogenic bladder, by controlling detrusor response. With tripolar stimulation it is possible to achieve a more physiological micturition with lower bladder pressure, at least in the laboratory. Also tripolar stimulation seems to prevent detrusor sphincter dyssynergia due to sphincter contraction during conventional stimulation. The ability to initiate bladder contractions without simultaneous external sphincter activity could be the answer to the high pressure micturition problem. However, there are still some questions to be answered before progress to a more widely use of these techniques. The cuff electrode needs some refinements: it ought to be reduced in size and structure to allow positioning the nerve within the electrode without the need of sectioned it. After change the electrode structure, further tests in animal model should be made to study the urinary flow and if a complete voiding is achieved during tripolar stimulation.

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CHAPTER 8 DISCUSSION

CHAPTER ONE

MICTURITION IN HUMAN

Chapter 1 - Micturition in Humans

Normal micturition involves coordinated function of the lower urinary tract, that is the bladder, urethra and urethral sphincter mechanism. The functions of the urinary bladder are storage and periodical expulsion of the urine. The main function of the urethra is to conduct the urinary flow to the exterior. The sphincter mechanisms are responsible for urinary continence.

During storage the urethral conduit remains closed by the urethral sphincter mechanism, preventing leakage of urine, while the bladder becomes progressively distended. When a certain capacity is reached, the bladder contracts and the urethral conduit is opened to establish flow of the urinary stream. During both phases of micturition the morphological arrangements of the ureterovesical junction prevent vesicoureteral reflux. Even in the storage phase, the ureterovesical junction remains closed unless a peristaltic wave occurs and opens it, forcing urine in to the bladder. As the bladder fills and distends, the trigone is progressively stretched, increasing the resistance to the intramural ureter which firmly close, avoiding urinary reflux to the kidneys.

The efficiency of the micturition process relies on the perfect arrangement of the smooth musculature of the bladder, urethra and ureterovesical junctions and also in the striated musculature of the urethral sphincter. However, there is still controversy about some of the important factors relating to micturition and the real functions of the different structures of the lower urinary tract in the process.

1.1 - Anatomy

The muscular anatomy of the lower urinary tract is better understood after many detailed dissection studies. Elementary studies such as the ones published by Wesson, 1920; Hunter, 1954; Lapides, 1958; Gil Vernet, 1968 and Elbadawi, 1973 are the basis for these modern anatomical concepts. Although there is a general agreement about the muscular components of the lower urinary tract, opinion on the anatomical division of the bladder and urethral sphincter has changed.

The major component of the bladder wall is smooth muscle, the detrusor. Under the conventional view, the detrusor is arranged in three layers: a middle circular layer surrounded by an outer and an inner longitudinal layer (Ross et al, 1989). Nowadays, it is accepted that the bladder musculature is divided into three coats only at its outlet. Elsewhere, the detrusor is formed by relatively coarse muscle bundles, widely separated and with muscle fibres moving freely between layers (Tanagho, 1992). In the older textbooks, the bladder was anatomically divided into three parts, the apex, the corpus and the cervix. However, these divisions were not well related to bladder function. More recently, the idea of dividing the bladder wall into the detrusor and trigone is supported by Wein et al (1991). The trigone is the posterior region of the bladder wall between the two ureteral orifices and the vesico-urethral junction. This area can be further divided into superficial and deep trigone, the deep trigone being a continuous part of the detrusor muscle (Tanagho and Pugh, 1963, Elbadawi, 1982, Gosling and Chilton, 1984, Tanagho, 1992).

There is also controversy about what happens after the vesico-urethral junction. The conservative view describes the urethra as a

passive conduit to the urinary stream, composed by smooth muscle. The urethra in males is much longer than in females. It is divided in to 4 portions. The first portion, the prostatic urethra, is rich in elastic tissues which helps to maintain continence during the storage phase of micturition. The external urethral sphincter, which promotes voluntary continence, embraces the membranous urethra.

Nowadays, it is more accepted that the sphincter mechanism involves practically the entire length of the urethra in the female and the entire prostatomembranous urethra in the male. According to Woodburne (1968), the detrusor fibres converge to form the bladder neck, where the middle circular layer ends. The outer layer becomes spiral as it extends down in the female urethra, acting as a physiological sphincter. In males, a similar configuration occurs at the distal end of the prostatic urethra. This theory was already described by Lapides in 1958 and is shared by Tanagho et al, 1968. According to these authors, the bladder neck and the smooth muscle spiral in the proximal urethra have an important function in regulating the urinary flow from the bladder to the urethra and control continence. Moreover, smooth muscle extends throughout the entire length of the female urethra which is regarded as having an active smooth muscle sphincter mechanism (Wein et al, 1991). However, there is still much discussion about urethral sphincter mechanisms, with considerable dispute over the existence of an anatomical internal sphincter. Tanagho (1992) denied the existence of any sphincteric muscular arrangement surrounding the bladder neck. Woodburne (1967) had found arching fibres at this level, but concluded that they arch away from the bladder neck. Rather, a physiological internal sphincter which consists of a portion of the posterior urethra and the bladder neck seems to be more accepted (Lapides, 1958; Tanagho and Schmidt, 1966; Wein and Raezer, 1979, Wein et al, 1991).

Elbadawi (1988) proposed a third way of dividing the lower urinary tract in mammals, based on embryological, anatomic, neuromorphologic and pharmacological studies in animal model and humans (Elbadawi, 1973 and 1983). He proposed that the muscular apparatus responsible for micturition should be divided into four functional units. These units include three smooth and one striated muscular components. The smooth muscle components are called the bladder body detrusor, the lissosphincter and the ureterotrigonal muscle. The striated component is mainly the rhabdosphincter. He divides the bladder functionally into body (the body detrusor model described by Hunter in 1954) and base. The base is the part of the detrusor that lies below the ureters entrances. The lissosphincter comprises the bladder base detrusor, its caudal extension into the urethra and its cranial extension to the vesical end of the ureters. The organisation of the muscle bundles will provide a sphincter mechanism at this point. The striated muscles related to the urethra include the urethral rhabdosphincter and the periurethral muscle, which is part of the pelvic diaphragm and corresponds to the urethral external sphincter.

Evolution has placed a number of structures in and around the lower urinary tract to assist continence. In addition to the structures described above, there are extrinsic structures which are important in the continence mechanism, such as the connective tissue support and the muscular support (levator ani). The urethrovesical junction is well supported by the pubocervical ligaments in women, or by the puboprosthatic ligaments in men. Problems with support of the proximal urethra and vesical neck are frequent cause of stress urinary incontinence. These problems are related to the anatomical position of the vesical neck. In normal standing women, the vesical neck is seen above the attachment of the puborectal ligament to the pubic bones, this

position changes based upon resting and voiding. Fluoroscopy studies show that the contraction of the levator ani can elevate the vesical neck, indicating that the levator ani has a function in controlling the urethral support (De Lancey, 1990). These later anatomical relationships are quite different in children. Although the adult bladder lies behind the pubic symphisis and is largely a pelvic organ, in children it is usually situated higher.

1.2 - Innervation of the Lower Urinary Tract

The innervation of the lower urinary tract is complex and not yet fully understood. Extensive studies have led to the identification of several areas in the spinal cord and brain that exert neural control on the act of micturition. There are components from the autonomic and from the somatic nervous systems.

The efferent innervation of the smooth muscle from the bladder and urethra is under influence of the sympathetic and parasympathetic divisions of the autonomic nervous system. Moreover, the efferent innervation of the external urethral sphincter is under influence of the somatic nervous system (Bradley et al, 1974, Duckett et al, 1976, Nyberg-Hansen, 1966).

Afferent axons also have been observed in the lower urinary tract (Gosling and Dixon, 1974). They are small thinly myelinated and unmyelinated C axons which are sensitive to the impulses in the bladder wall and also to pain impulses from the trigone which are thought to travel in the hypogastric nerve, as described by de Groat, 1975.

The sympathetic nervous system originates from the last spinal thoracic (T_{10}-T_{12}) and first two lumbar segments. Their preganglionic axons end on postganglionic neurones located near the aortic plexus. Noradrenaline is the sympathetic transmitter substance between the postganglionic neurone and the effector cell. However, in recent years the principle that one nerve releases only one neurotransmitter has been questioned (Burnstock, 1976, Chan-Palay, 1984).

The parasympathetic nervous system supplies the pelvic viscera via the pelvic branches of the second to fourth sacral spinal nerves. The long axons of the preganglionic parasympathetic neurones end on short postganglionic neurones located near the visceral structure. Acetylcholine is the chemical mediator at the parasympathetic synapses. However, sympathetic and parasympathetic nerve terminals are often close to each other as well to the muscle cells. So, functional interactions between nerves are possible. For example, noradrenaline released from sympathetic nerves acts as a pre-junctional modulator on acetylcholine release from parasympathetic nerves (Burnstock, 1986).

Parasympathetic responses are more localised and shorter than sympathetic ones. Strong sympathetic stimulation produces diffuse effects. The preganglionic neurones in the sympathetic nervous system synapse with many postganglionic neurones. These postganglionic neurones will supply different effector cells, allowing more divergence of stimulus.

Both the parasympathetic and sympathetic nerves which supply the bladder contain non cholinergic, non adrenergic nerve fibres. These nerve fibres are generally known as NANC nerves - i.e. Non Cholinergic, Non Adrenergic (Anderson, 1986; Brading et al, 1986). It has been proposed that ATP is the neurotransmitter utilised by the NANC nerves (Anderson and Sjögrem, 1982, Kasakov and Burnstock,

1983). However, the lack of response to specific adenosine antagonist (Dean and Downie, 1978) and the persistence of responses to field stimulation following tachyphylaxis to ATP (Ambache and Zar, 1970) do not support this proposal. According to Creed et al, (1983) the mechanisms involved in the NANC neuro-effector transmission and the identification of transmitter substances remain unsolved.

The external urethral sphincter is under the influence of the somatic nervous system. The somatic also has afferent components, central integration stations and effector pathways. It is also organised in reflex arcs. The somatic is largely under voluntary control.

Motor-neurone axon terminals are commonly considered to release acetylcholine. In contrast to autonomic axons, the motor axon can only stimulate the muscle. If an inhibition of activity is desired, as in the external urethral sphincter, this must be an inhibition of the motorneurons rather than by a direct inhibition of muscle fibres.

It is known that the majority of autonomic nervous system axons are thinly myelinated slowing-conducting group C axons. However, the somatic axons are faster conducting. Thus, it is expected that the external sphincter reacts faster to stimulation than the bladder. However, the normal functioning of the lower urinary tract depends on co-ordinated action of the sphincter mechanisms and the bladder. Thus, the ideal stimulation should allow the autonomic and somatic motor system to work co-operatively for appropriate function.

1.3 - Viscoelastic Properties of the Bladder and Urethra

Bladder and urethra viscoelastic properties are important clinically because they allow derivation of several parameters related to the structure and function of these organs. However, it is important to remember that these measurements depend on the physiological environment where they were made.

Two basic factors may influence the pressure recorded inside the bladder and urethra: a neuromuscular factor and a mechanical factor. The neuromuscular factor is related to the general characteristics of live tissues and includes neural input mechanisms, cellular membrane depolarisation and active muscular stretch. The mechanical factor is related to structure of the muscular tissue, mainly its collagen component, which is quite abundant in the bladder but also in the urethra.

The viscoelastic properties of the bladder will interfere directly in the bladder compliance, which is the change in bladder volume associated with the changes in bladder pressure. When the bladder is filling, it is subject to a certain strain (deformation of a solid related to force per unit of area). The measurements of this strain can be fitted in a curve: the stress curve. This curve can predict bladder compliance. However, the properties of bladder components, i.e., smooth muscle, collagen and elastin are not predicted in these curves and may interfere in these measurements. Fung (1981) proposed to study the morphology of the organ by means of anatomy, histology and ultrastructure of the tissues to define the architecture and constituent elements. Then, establish the mechanical properties of the materials involved. However, these measurements are limited because live tissues are subject to significant deformation *in vitro*.

The human bladder wall contains 54% of insoluble protein and less than 43% of muscle elements. Because of the mechanical nature of these constituents, the bladder may distend up to 300% volume, which is ideal for a storage organ. The normal bladder can adapt to increasing volumes with minimum increase in pressure. However, external factors can interfere in these properties, such as rapid filling, outlet obstruction, bladder decentralisation, chronic infections and ageing (Susset and Regnier, 1986).

The urethra has structural similarities to the bladder mainly in its components: smooth muscle, collagen and elastin. However, geometrically it is different from the bladder. Because the urethra is not a storage organ, its distensibility is limited. The urethra carries a flow of urine during micturition, so its properties are frequently approached from the fluid dynamic point of view, which apply several techniques based on pressure volume studies during voiding. One of these techniques is the measurements of the urethral pressure profile as proposed by Brown and Wickham.

There are also external factors that can interfere with these results, such as the size of the urethral catheter. Regnier et al (1983) measured the urethral compliance in young nulliparous women with different sizes of catheters. The measurements were made at the urethral sphincter level. The results showed that little increase in urethral pressure occurs for the size range of 5F to 25F. With urethral catheters wider than 25F, however, the urethral wall reached its elastic limit and the sphincter contracts, resulting in higher pressures.

Abnormal urethral compliance is also related to incontinence mainly in woman, ageing, urethral rigidity due to chronic infection and urethral stenosis.

1.4 - Control of Micturition

The bladder is controlled by the cerebral control centres, the cerebellar control areas of micturition, the control medullary centres and the centre of micturition in the spinal cord. The healthy bladder can be stretched to a certain volume almost without increase in its pressure. Once the capacity is reached, mechanoreceptors located in the bladder wall are excited by the stretch of the bladder. Their signals are transmitted to the micturition centre by the afferent axons of the hypogastric nerve. There, they are used in different circuits, including those related with the motor response. The sympathetic axons of the hypogastric nerve are considered to regulate bladder compliance and urethral resistance to the urinary flow.

From the brain stem, efferent axons cross all the way back to the micturition centre in the sacral cord. This centre controls the micturition reflex via the parasympathetic axons of the pelvic nerve. It triggers the bladder to contract. Thus, the detrusor contracts, the intravesical pressure rises and the bladder neck opens. The micturition can be initiated but may be deferred and the urine stream can be interrupted by voluntary control of the external sphincter. Figure 1 shows a diagram of the neurologic control of the lower urinary tract.

The spinal cord is also very important in controlling the process of micturition. It has a complex internal structure. Mostly afferent or sensory axons enter the spinal cord through the dorsal roots of the spinal nerve, and mostly efferent or motor axons leave by the ventral roots - Law of Bell and Magendie (Magendie, 1822). However, afferent axons have been found in the ventral root in contrast to the traditional view (Coggeshall, 1980).

Garry et al (1957 and 1959), studied the reflex involving the external urethral sphincter in a classical work in cats carried out in Glasgow. The electrical activity of the striated urethral sphincter was recorded as the bladder filled. The external sphincter and the bladder were still active in decerebrate animals. However, spinal transection at the lower thoracic level stopped evoked reflex contractions in the bladder and activity in the external sphincter.

The spinal cord nuclei which control the smooth muscle of bladder and urethra and the urethral external sphincter are located in the lumbosacral region. Their exact locations can vary in different species. In man the sympathetic autonomic nucleus is located in the intermediolateral grey matter column of T_{10}-T_{12} segments and the parasympathetic autonomic nucleus in the same column of the S_2-S_4 segments. The efferent somatomotor innervation of the urethral rhabdosphincter also originates in the S_2- S_4 segments. Studies reveal that the motorneurons of the urethral sphincter have now been localised specifically in the Onuf's nucleus. This nucleus was described by Onufrowicz (1902) and it can be found near the sacral parasympathetic nucleus (Elbadawi, 1988).

It is generally accepted now that a specific region in the pontine-mesencephalic reticular formation is the main cephalic control centre of micturition. This centre exercises facilitatory or inhibitory control of the spinal cord micturition centre (Blaivas, 1982; De Wall et al, 1984).

The peripheral axonal projections of the dorsal spinal root ganglia are represented by nerve fibres carrying afferent impulses from the lower urinary tract. They respond to the same segments as the sympathetic, parasympathetic and sphincteric spinal cord nuclei. The central projections of these ganglia enter the spinal cord by the dorsal roots of the corresponding spinal nerve. After entering the spinal cord,

Afferent and efferent neural pathways of micturition

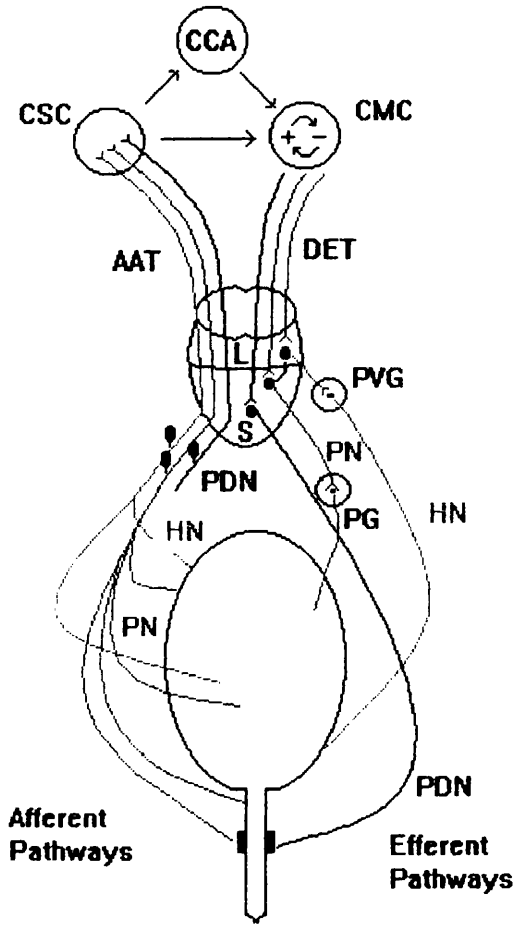


Figure 1: Neurological control of the lower urinary tract. Modified from Elbadawi, 1988.

Key:

CCA: cerebral and cerebellar control area of micturition; CMC: control medullary centre

CSC: cerebral sensory centre

AAT: ascending afferent (sensory) tract; DET: descending efferent tract

L - lumbar column; S - sacral column

Nerves: HN - hypogastric nerve, PN - pelvic nerve, PDN - pudendal nerve

Ganglia: PVG - paravertebral sympathetic ganglia, PG - pelvic ganglia

some afferent fibres synapse with the corresponding spinal nucleus of micturition to form segmental reflex arcs.

The hypogastric nerve and the pelvic nerve contain respectively the peripheral efferent sympathetic and parasympathetic innervation of the vesicourethral smooth muscle and internal genital organs. The peripheral axonal projections of the neurones of the sympathetic spinal nucleus carried by the ventral roots of the spinal nerves are connected to the segmental corresponding ganglia of the lumbar sympathetic chain. In humans, fibres of these ganglia connect to the superior hypogastric plexus, which bifurcates to form the right and left hypogastric nerves (Kuntz, 1944; Elbadawi, 1983). The peripheral axonal projections of the neurones of the parasympathetic spinal nucleus are conducted to the bladder and urethra by the ventral roots of sacral spinal nerves and the pelvic nerves. The hypogastric and the pelvic nerve at each side link away from the urogenital organs, branch repeatedly and fuse to form the pelvic plexus or inferior hypogastric plexus.

It is accepted that the efferent innervation of the urethral external sphincter is conveyed by the pudendal nerve. However, recent studies in dogs demonstrate that the urethral external sphincter receives somatomotor innervation by both pudendal and pelvic nerves (Morita et al, 1984).

The sensory (afferent) output of the bladder and urethra originates largely in the region of the bladder neck and it is conveyed by the hypogastric nerve to the lumbar segments and by the pelvic nerve to the sacral segments of the spinal cord.

In summary, the storage and periodic elimination of urine are dependent upon the activity of two functional determinants - the maintenance of the urinary bladder as a reservoir and the urinary flow resistance by the bladder neck, urethra and striated sphincter. These

structures are controlled by three different groups of peripheral nerves, which must work in harmony: the thoracolumbar sympathetic, the sacral parasympathetic and the sacral somatic.

The thoracolumbar sympathetic is represented by the hypogastric nerve and sympathetic chain. It is believed to promote the storage of urine by inhibition of the detrusor muscle, excitation of the bladder neck and modulation of cholinergic transmission in bladder parasympathetic ganglia.

The sacral parasympathetic is represented by the pelvic nerve. It provides the major excitatory input to the bladder, which is mediated by cholinergic and non-cholinergic transmitters (de Groat et al, 1982, 1990). Its excitation makes the detrusor contract and the bladder neck relaxes.

The sacral somatic nerve is represented by the pudendal nerve. The pudendal nerve is important to innervation and control of the urethral external sphincter (Juenemann et al, 1988).

CHAPTER TWO

GENERAL ASPECTS OF DYSFUNCTION IN THE MICTURITION PROCESS

Chapter 2 - General Aspects of Dysfunction in the Micturition Process

Disturbances of micturition are major problems. The aetiology may vary according to the sex or age of the patient, but is almost always due to either anatomical problems related to the lower urinary tract or neurological dysfunction. Most anatomical problems can be solved by using appropriate surgical techniques, which are beyond the scope of this research. However, voiding dysfunction due to neurological disorders remains difficult to treat. The long term aim of the present research is to find a better treatment for the disturbances of micturition due to neurological dysfunction.

Despite the fact that there has been a great deal of good research in this field, there are still several points to be clarified. The control of normal micturition is complex and involves interaction of several neurological pathways. Any disturbance in these pathways may produce a disorder of micturition. Neurogenic bladder is a general name for all these uropathies developed as a consequence of neurological lesions. It is usually a complex malfunction which in most cases acts as an obstructive factor that causes urinary incontinence and urinary tract damage due mainly to disturbance of the voiding mechanism leading to high bladder pressure and urinary tract infections. In these cases, the stagnant urine in the bladder predisposes to urinary infection and overflow incontinence. Another serious problem is the development of high pressure in the lower urinary tract, with consequent reflux to the ureters and kidneys. The most dangerous consequences are hydronephrosis and renal failure, uraemia and ultimately death. However, urinary incontinence is the most common manifestation of neurogenic bladder.

Treatment for neurogenic bladder has been improved in the last 30 years. In the past, the majority of patients with neurological diseases had a short life. They died due to complications inherent to the neurological disease itself or due to post-surgical problems such as infections. The patients who survived were usually isolated from normal social life. So, any associated urinary incontinence was an unreported problem and because of this the importance of research into this treatment was not fully realised for a long time.

With the progress of medicine and science leading to the advent of more sophisticated equipment and new surgical techniques, the treatment for these neurological patients improved their prognosis. Also, the utilisation of urodynamic evaluation helped in achieving better results, since it permits diagnosis and more appropriate treatment for each individual patient. Urodynamic is a neurourologic diagnostic tool related to individual identification and measurement of physiologic and pathologic factors involved in the storage and evacuation of urine. More recently, videourodynamics have been used for evaluation of complex lower urinary tract problems. It is a technique which permits synchronous urodynamic tests and cystourethrography by means of filling the bladder with radiographic contrast material to allow urodynamic tests and periodical screening of the lower urinary tract.

Neurogenic bladder can be classified using urodynamic. When exact urodynamic classification is possible, this system provides a truly exact description of the micturition dysfunction (Barrett and Wein, 1991, Wein, 1992). Urodynamic classification was first proposed by Krane and Siroky, in 1979, and it was reviewed by them in 1984. There are other classification systems in use such as the classification system proposed by the International Continence Society (Abrams et al, 1988, Wein et al, 1992). This classification is based on the dynamics and the

interaction between bladder and urethral sphincter during the storage and voiding phase. Both are presented in table 1.

Patients with detrusor-sphincter dyssynergia are more difficult to treat and they have more risk of damage to the urinary tract. In these cases, the hypereflexia makes the bladder contract more frequently against a closed sphincter mechanism. Thus the pressure in the renal system rises, with risk of ureteral reflux and hydronephrosis with consequent irreversible kidney damage.

There are distinct therapies to treat patients suffering from neurogenic bladder and specific treatments can be used to facilitate bladder emptying or to facilitate urine storage. Generally, the treatment starts with drugs, training, clean intermittent urethral catheterization and manoeuvres to help voiding. As an example, patients with detrusor hypereflexia and sphincter dyssynergia can be treated satisfactory with anti-cholinergic drugs and clean intermittent catheterization. The anti-cholinergic drug will attenuate detrusor contractions and decrease the bladder pressure, while the intermittent catheterization will empty the bladder more effectively. Sometimes, self-catheterization becomes difficult, mainly in the elderly and infirm patients, and tetraplegics with inadequate home support. In these cases, indwelling urethral catheterization is a practical option of bladder drainage but requires conscientious catheter care with regular changes and careful fixation (Tan and Edmond, 1994). However, despite correct clinical treatment, some patients do not improve and further surgical intervention may be necessary (Bauer and Joseph, 1990; Costa Monteiro and D'Ancona, 1991). There are several surgical techniques that can be used to treat or attenuate the symptoms of neurogenic bladder. External sphincterotomy and resection of the bladder neck have been conventional treatments for chronic retention in paraplegic men. It is a permanent way to relieve

Urodynamic Classification	
Detrusor hypereflexia	Detrusor areflexia
Coordinated sphincters	Coordinated sphincters
Striated sphincter dyssynergia	Non relaxing striated sphincter
Smooth sphincter dyssynergia	Denervated striated sphincter
Non relaxing smooth sphincter	Non relaxing smooth sphincter

International Continence Society Classification	
Storage Phase	Voiding phase
Bladder function	
Detrusor activity Normal Overactive Unstable Hypereflex	Detrusor activity Normal Underactive Acontractile
Bladder sensation	
Normal Increased or hypersensitive Reduced or hyposensitive Absent	
Bladder Capacity	
Normal High Low	
Compliance	
Normal High Low	
Urethral Function	
Normal Incompetent	Normal Obstructive Overactive Faulty mechanisms

Table 1 - Two ways of classifying micturition dysfunction. The classification based on the urodynamic evaluation (Krane and Syroky, 1984) and the classification proposed by the International Continence Society (Abrams et al, 1988).

obstruction, but will cause permanent incontinence. As an option, vesicostomy can provide a temporary relief of obstruction in the system, but the incontinence still persists. However, many patients prefer to take the risks associated with large residual volumes and high voiding pressures in order to remain reasonably continent. More recently bladder augmentation has been used to solve the problem of capacity and to give continence and stability to the renal system (Sidi et al, 1990). Nevertheless, it is necessary to use a healthy stomach or intestine to enlarge the bladder and it demands a long hospitalisation, which associated bladder augmentation with high morbidity and high cost.

Therefore, in some cases, untreated incontinence remains as a substantial social and clinical problem. An impressive amount of money has been spent on research to find a more satisfactory approach for all patients, but until now, there is no treatment which could solve all of the cases. It is clear there is a need to find more effective therapies for these cases.

Electrical stimulation of motor axons supplying the bladder and urethral sphincter has been widely investigated both clinically and experimentally as an alternative treatment to restoration of lower urinary tract function, mainly in patients that suffer spinal cord injury. According to Tanagho, 1988, a genuine interest in the electrical control of bladder function began in 1950. The first reports of attempts to initiate micturition through implantable electrodes were published (Timm and Bradley, 1957; Boyce et al, 1964; Hald, 1969; Ingersoll et al, 1969). Since then, various kinds of electrical implant have been tried. These include electrodes implanted in the pelvic nerve (Holmquist, 1968), in the spinal cord (Jonas and Tanagho, 1975), in the sacral root (Brindley, 1982) and intravesical (Madersbacher, 1990). However, the initial results were discouraging and the use of implantable electrodes in

urology remained at the experimental stage (Schmidt et al, 1979). The early attempts to use electrical stimulation failed to produce complete bladder evacuation and to control urinary incontinence and urinary infection (Bradley et al, 1971, Schmidt, 1986). But the encouraging results achieved with cardiac pacemakers and the treatment of pain by electrostimulation continued to motivate new research in the urinary field.

Four potential sites of stimulation for micturition control have been described more frequently: the spinal cord, the detrusor, the pelvic nerves and the sacral roots. Each site has been reported to offer different advantages (Schmidt et al, 1979 and 1986, Brindley et al, 1982, Tanagho et al, 1988, Madersbacher et al, 1990, Walter et al, 1990, 1992, Sawan et al, 1992). In addition, pudendal nerve stimulation may be used mainly when the control of the external urethral sphincter is required. Pudendal nerve stimulation can be applied also in patients with severe reflex incontinence but normal or nearly normal sensation in the sacral dermatomes (Brindley, 1990). A summary of the advantages and disadvantages of each method is listed in Table 2.

Direct electrical stimulation of the bladder was most effective in patients with hypotonic and arreflexic bladders. Initial success, defined as low urinary residual and sterile urine, was achieved in 60% of the patients. However, a failure in response usually follows, associated with electrode mal-function. Also, the spread of current to neighbouring structures with lower excitation threshold caused abdominal, pelvic and perineal pain. Other problems included concurrent desire to defecate, contraction of the pelvic and leg muscles and erection and ejaculation in men. Moreover, the increase in intravesical pressure was not frequently followed by bladder neck opening, so other methods to permit

micturition were necessary (Merril, 1974, Halverstadt and Parry, 1975, Barrett and Wein, 1991).

Advantages	Disadvantages
Direct bladder stimulation	
• easy placement of electrodes • high specificity • direct application in lower motor lesions	• electrode malfunction due to movements during micturition • production of fibrosis • bladder erosion • higher current needed to stimulate • poor bladder contraction
Pelvic nerve stimulation	
• direct control to detrusor	• low toleration to stimulation • risk of permanent nerve damage • pain reflex
Spinal cord stimulation	
• minimise nerve injury due to electrode movements	• concurrent stimulus of urethral sphincter • pain reflex • surgical risks of nerve damage
Sacral root stimulation	
• allow separation of detrusor and sphincter response • minimise pain reflex • minimise nerve injury due to electrode movements	• surgical risks of root damage
Pudendal Nerve Stimulation	
• direct control of sphincter response • can be used to inhibit reflex detrusor contractions.	• risk of impotence, faecal and urinary stress incontinence due to nerve damage. • pain reflex
	• uncomfortable devices are needed (Brindley, 1990)

Table 2: Comparison between different sites of stimulation used to attempt to control the lower urinary tract.

Sacral nerve roots seem to be highly appropriate as a site for stimulation when the parasympathetic efferent innervation to the detrusor is intact. The anatomy of the sacral roots allows the separation of sensory and motor roots, so stimulation can be restricted almost completely to the efferent axons. It could avoid the concurrent stimulation of afferent axons which cause unwanted effects such as pain.

Pain has been one of the greatest restrictions to sacral root stimulation. Posterior rhizotomy can be performed to exclude the afferent innervation in specific cases, when the collateral effects caused by this surgical approach become irrelevant for the patient's life.

Ventral sacral root stimulation may allow separation between detrusor and urethral sphincter response, minimises pain and offers lower risks if compared to the spinal cord surgeries. The possibility of nerve injury due to electrode movement is also reduced because it is easier to fix the electrode in the small vertebral space (Schmidt, 1986).

However, another limitation of more widespread application of sacral root stimulation lies in the recruitment characteristics of the electrodes that cannot selectively activate the smaller motor axons supplying the bladder wall. Thus bladder contraction is always accompanied by contraction of the external urethral sphincter, causing elevated pressures in the bladder, with high risk of kidney damage (Jonas and Tanagho, 1975, Schmidt, 1988, Brindley, 1990, Hohenfellner et al, 1992, Creasey, 1993, Haleem et al, 1993, Mostwin, 1993).

Brindley pioneered a novel approach to bladder control problems using sacral root stimulation (Brindley et al, 1972 and 1973). As early as 1969, Brindley began to test sacral root stimulation to control the lower urinary tract. He and his co-workers developed an experimental model in baboons using sacral root stimulation through implantable electrodes to try to establish a technique for blocking axons through the implant

(Brindley, 1972 to 1980). The implant consisted of three electrodes. This tripolar arrangement was employed to ensure that stimulating currents did not spread outside the electrode and stimulate unwanted structures. Also, it can be used to provide selective stimulation of small myelinated axons. This technique has been one of the most successful applications of functional electrical stimulation. It has been used in patients since 1982 in Britain (Brindley et al, 1982) and world-wide (Kerrebroeck et al, 1993) and has restored significant functional control of bladder to many hundreds of patients in several countries. However, in most of patients, the external urethral sphincter was activated during detrusor stimulation, preventing a more physiological micturition to occur. The large myelinated axons to the sphincter were still stimulated with lower current than the small myelinated axons that innervate the detrusor. So, the urethal external sphincter was stimulated and closed before the detrusor contracts.

To avoid this recruitment sequence problem, the urethal sphincter should be inactivated. Brindley proposed three possibilities. The first would be to fatigue the sphincter and then stimulate the detrusor. The current needed would be higher, because the external sphincter is rather resistant to fatigue. Another possibility is to apply strong stimulation in bursts. After stimulation stops the detrusor stays contracted for longer than the urethral sphincter. However, the micturition will occur in bursts, that sometimes is not sustained long enough to void the bladder completely. A third option would be blocking anodally the large myelinated axons to the sphincter without blocking the small axons that will innervate the detrusor.

Pudendal nerve block can also be used to relieve the obstruction at the level of the external sphincter. However, bilateral lesion of the pudendal nerve may cause impotence, faecal and urinary stress

incontinence (Wein et al, 1976, Barrett and Wein, 1991). Another point of controversy is how the somatic neural innervation reaches the striated musculature of the external urethral sphincter. The classical neurological view is those motor neurones in the sacral ventral horn innervate the external urethral sphincter via the perineal branch of the pudendal nerve (Fletcher and Bladley, 1978). However, other groups believe that some or all the somatic innervation of the external urethral sphincter travels via pelvic nerve (Gil Vernet, 1964, Donker et al, 1982, Gosling et al, 1982, Morita et al, 1984). The importance of the pudendal nerve has been demonstrated in more recent studies, where pudendal blocks and neurotomies were performed (Schmidt, 1986).

Although solutions exist for the recruitment problem caused by conventional sacral root simulation, the ability to initiate bladder contractions without simultaneous external sphincter activity, so avoiding detrusor-sphincter dyssynergia, would be a better answer to the problems caused by high pressure into the bladder and renal system.

The present study used acute animal experiments to evaluate a tripolar stimulation strategy based on a computational model (Fitzpatrick, 1991) which predicts that it is possible to activate selectively small autonomic motor axons whilst blocking excitation in larger skeletomotor axons. An additional prediction is that it should be possible to produce unidirectional action potentials. The work in this thesis was developed to study the viability of the above predictions as a potential answer to the problem caused by the conventional recruitment sequence in the lower urinary tract. In the realisation of this work, the experiments were divided into two main parts. First of all, the electrode and the stimulator were tested in a peripheral nerve preparation. The ideal parameters for stimulation and blocking were observed, and the devices were improved to be used in the animal model. Then, the

possibility of using this tripolar stimulation strategy to stimulate the bladder was evaluated in the animal model. The rabbit was chosen as the experimental animal. Details of the modelling studies will be presented in chapter 4.

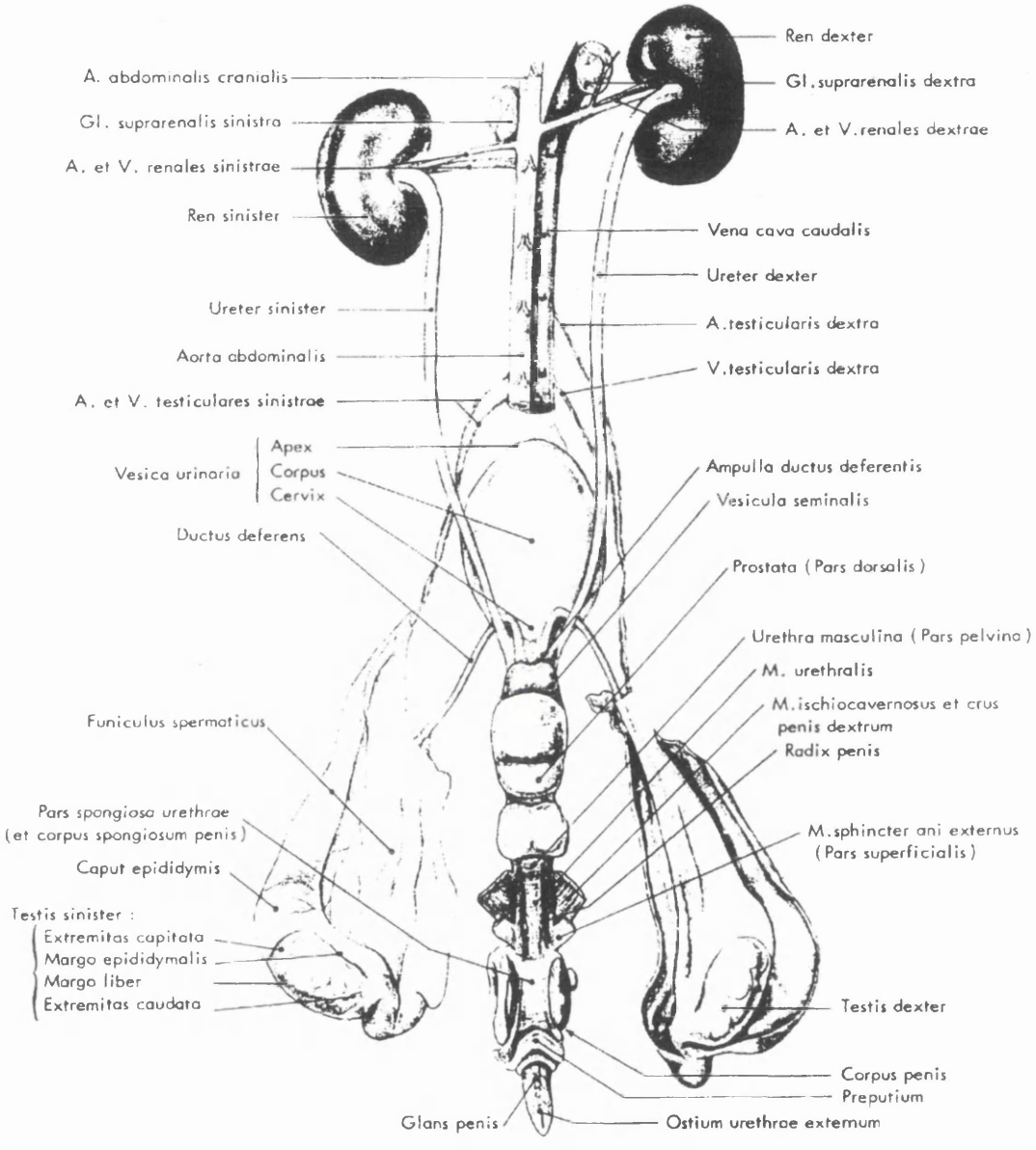
CHAPTER THREE

RABBIT AS A MODEL

Chapter 3 - Rabbit as a Model

The anatomical descriptions in chapter 1 refer to human anatomy, but there are many similarities between the urinary tract in mammals (Levin et al, 1994). Nevertheless, the lower urinary tract can present particularities in each species. Although it is beyond the scope of this work to describe in detail these particularities, they should be taken into account when using these mammals as animal models in urological projects.

There are four major mammal species utilised for urological research proposes: the dog, the cat, the rat and the rabbit. Each has specific advantages and disadvantages for use (Levin et al, 1988). Although the cat and dog's urinary bladders are more similar to humans, the high cost of these animals and their house requirements have prevented their use more widely in research. Rabbits have been used as an animal model for several urological studies (Bradley et al, 1967; Creed et al; 1983; Kato et al, 1988, Kitada et al, 1989; Levin et al, 1981; 1987 and 1994; Wendt et al; 1983). The urinary bladder of rabbits is a thin-walled structure and lacks in abundant connective tissue, but its size is generally well suited for urological investigations. Also, the junction of bladder base and urethra is gradual and almost imperceptible in rabbits (Elbadawi, 1988). During this research, basic histological and urodynamic studies were performed on rabbit bladder and urethra to compare with human anatomy, and to determine the appropriateness of this species for the present study. These studies will be presented later. Figure 2 shows a rabbit urinary tract.



ORGANA UROPOETICA ET GENITALIA MASCULINA (FACIES DORSALIS).

Organes urinaires et génitaux du mâle (vue dorsale).

Male urogenital organs (dorsal aspect).

Figure 2 - Urinary tract of a male rabbit (after Barone et al, Atlas of Rabbit Anatomy)

Rabbits have been used as research models in several ways: *in vivo* whole animal preparation, *in vitro* bladder-strip, *in vitro* whole-bladder preparation, tissue homogenates or subcellular membrane preparation and histological and histochemical preparation (Levin et al, 1988).

In this present research, a whole animal preparation was necessary to observe the effect of sacral root stimulation on the bladder and also on the urethral sphincter mechanism. It is not only the contractile response that is important, but the interaction between the different structures related to the micturition process. Also, in vitro experiments were carried out in peripheral nerve preparations, and histological studies of the sacral root and peripheral nerves were made.

Levin et al, 1987, studied the contractile, functional and biochemical aspects of urinary bladder in rabbits. They reported that in the rabbit also, the micturition occurs due to a highly coordinated contraction of the bladder and the subsequent expulsion of urine through an adaptable shaped low - resistance urethra. The initial phase of bladder contraction, characterised by a rapid increase in pressure, resulted from cholinergic and purinergic stimulation. This initial phase of bladder contraction is supported by intracellular ATP. The next phase is a plateau, that maintains the pressure and evacuates the bladder (Levin et al, 1983). Most of the bladder volume is eliminated in this second phase. The ability of the bladder to maintain a contraction and empty is supported by aerobic metabolism. In the absence of oxidative phosphorylation, the bladder can not maintain increasd pressure (Levin et al, 1987).

There are several factors that could interfere with bladder response during experiments using rabbit as an urological animal model. Kitada et al, 1989, reported bladder contractions in rabbits in response to

perineal stimulation (somatovesical contractile reflex) and distal urethral constriction (rhythmic contractile reflex). Unlike the bladder contractions induced by perineal stimulation, the rhythmic contractions induced by urethral constriction were eliminated by spinal section. These responses must be taken into consideration during experiments where the bladder activity is evaluated.

The ability of the bladder to maintain a contraction and empty is lost immediately during anoxia. However, these abilities are regained after re-oxygenation (Levin et al, 1987). Acute in vivo overdistension of the rabbit urinary bladder for one hour resulted in a decreased in vitro contractile response of muscle strips to cholinergic and alpha-adrenergic stimuli ((Levin et al, 1983).

Several pharmacological studies have been developed in rabbits to see the effect of different drugs in the lower urinary tract (Levin et al, 1988 and 1991; Zderic et al, 1990). However, none of these drugs were used during the experiments in this research.

Outside the lower urinary tract, there are also other anatomical differences between rabbits and other mammals and humans, such as the spinal root distribution. They must be taken into consideration when a laminectomy is necessary. The lumbar and sacral vertebral column in a rabbit and the rabbit spinal cord are presented in figure 3 and 4. It has seven lumbar spinal nerves and five sacral roots. The first sacral root lies on the filum terminalis, with S_2 at the level of the cauda equina. The lumbosacral plexus is presented in figure 5.

Since some experiments were carried out in the sciatic and tibial nerve, the lateral aspect of the rabbit hind limb is presented in figure 6.

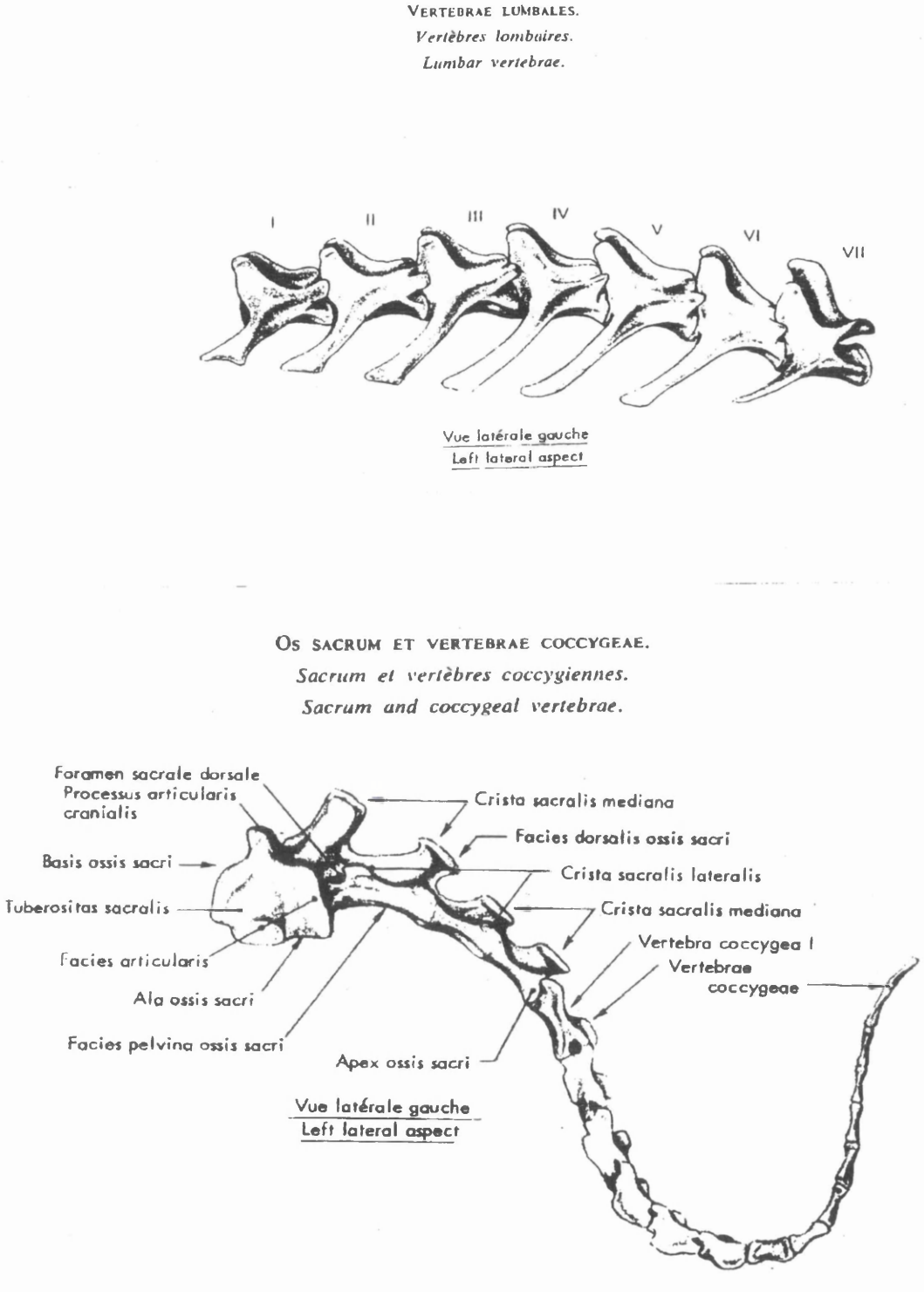


Figure 3 - The vertebral column of a rabbit showing the lumbar vertebrae, the sacrum (and sacral vertebrae) and the coccygeal vertebrae (after Barone et al (1973) - Atlas of rabbit anatomy)

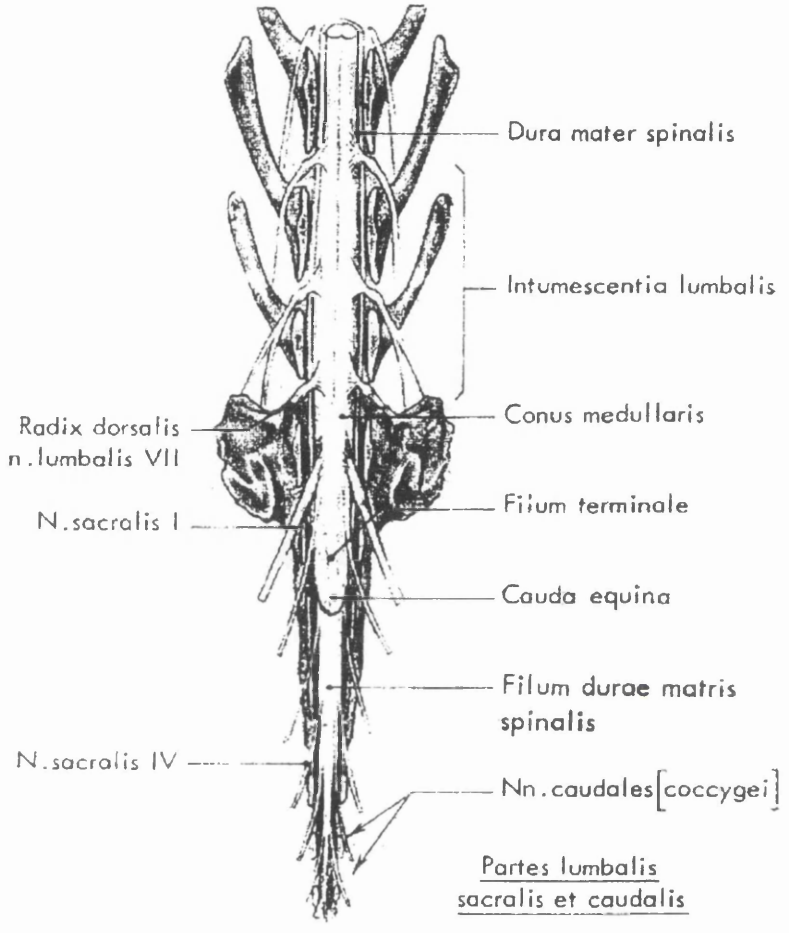


Figure 4 - Aspect of the spinal cord in rabbits. (after Barone et al (1973) - Atlas of rabbit anatomy)

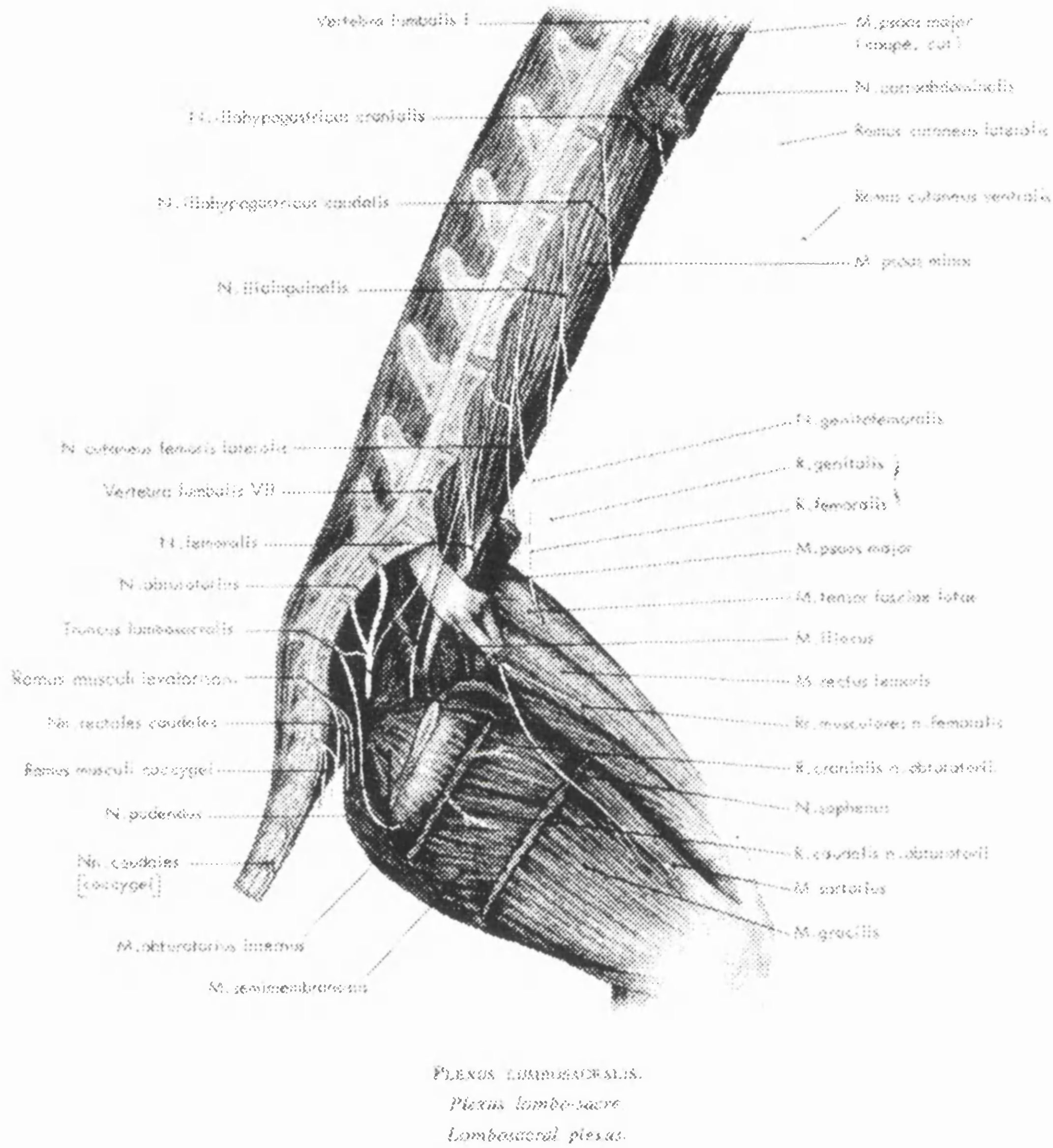
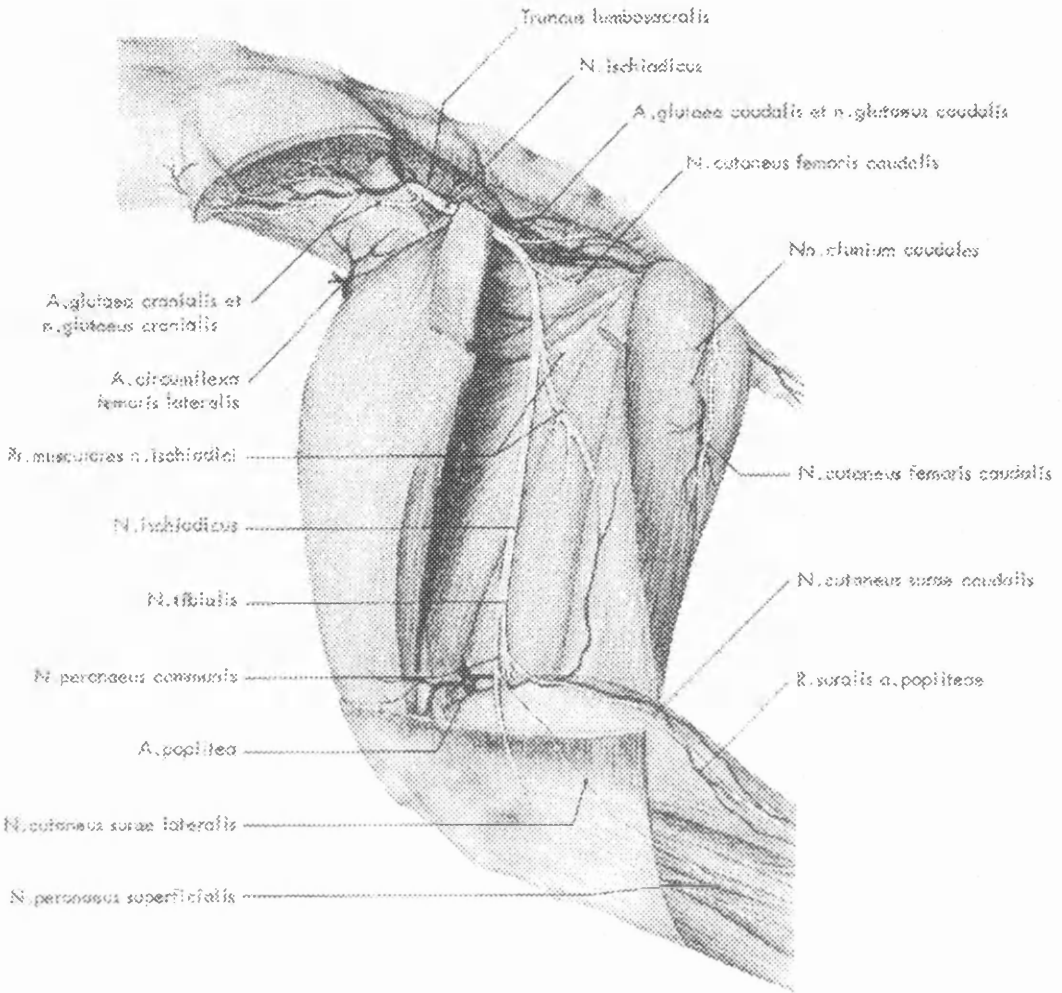


Figure 5 - Lumbosacral plexus in rabbits (after Barone et al, 1973)



NERVUS ISCHIADICUS.
Nerf sciatique.
Sciatic nerve.

Figure 6 - Lateral aspect of the rabbit hind limb showing the sciatic nerve and its branches (Barone et al, 1973)

CHAPTER FOUR

THE TRIPOLAR CUFF ELECTRODE AND MODELLING PREDICTIONS

Chapter 4 - The Tripolar Cuff Electrode and The Modelling Predictions

Electrical stimulation can be used to restore the function of the lower urinary tract. (Brindley, 1986; Schmidt, 1988; Mortimer, 1990). However, conventional bipolar stimulation electrodes cause action potentials to propagate in both directions away from the stimulus site. The ventral sacral root contains a mixed population of somatic motor and autonomic parasympathetic axons. Since larger somatic axons are excited at lower current thresholds than the smaller autonomic ones, it is impossible to activate the bladder without also activating the urethral sphincter. In the same way, it is difficult to prevent unwanted movement of skeletal muscles innervated by the same root. Thus, the attempts to control the bladder using sacral root stimulation have been limited by these recruitment characteristics. Conventional stimulation excites the skeletal muscle axons, so closing the external urethral sphincter before exciting the autonomic axons which initiate bladder contraction. Therefore, physiological voiding is impossible during stimulation. So, a better electrode is needed to improve stimulation by reversing these recruitment characteristics.

Recent modelling studies have developed a design of a tripolar electrode which could allow preferential activation of the small autonomic axons (Holsheimer et al, 1990; Fitzpatrick et al, 1991 and 1992; Wijkstra et al, 1991; Rijkhoff et al, 1992 and 1994). This research uses similar tripolar stimulation strategy as developed by Fitzpatrick, which will be described. The aim was to achieve total anodal block on the central direction and partial anodal block of only the faster somatic axons in the peripheral direction.

Tripolar electrodes consist of three ring electrodes placed within an insulated material. In the Fitzpatrick model, a cathode is asymmetrically placed between two anodes. The current will flow between the cathode and the anodes. The distance between the cathode and the anodes may interfere with the amount of current discharged. If the two anodal currents are equal and equally spaced from the cathode, an anodal block will occur in both directions. If the current through the proximal anode is reduced and the distance between this anode and the cathode is increased, it will be possible to generate an orthodromic action potential (Fitzpatrick, 1994).

4.1 - Fitzpatrick model

Fitzpatrick developed a mathematical model in order to predict the stimulus responses of myelinated nerve fibre membranes of different diameters to a tripolar stimulation strategy. His work is based in the model developed by McNeal (1976), which was designed to compute the threshold response of myelinated nerve fibres when stimulated by extracelular electrodes.

The design of the Fitzpatrick tripolar electrode relies on the relative distance between the cathode and the anodes and on the ratio of the anodal currents.

One aim of the model is to block the propagation of action potentials in all axons at the closest anode, thus, producing a total anodal block at this end of the cuff. At the farthest anode, however, action potentials continue to be propagated.

The other aim of the model is to maintain the total anodal block at the closest anode, but provide an anodal block of only the larger

diameter axons at the farthest anode whilst allowing the propagation of action potentials generated by the small diameter axons. These predictions are demonstrated in figure 7 A and B. During stimulation, all the axons are excited at the cathode. However, the small diameter axons in a nerve bundle have higher excitation thresholds compared to the large diameter axons (Blair and Erlanger, 1933). They also have higher anodal blocking threshold. By varying the ratio of anodal currents it may be possible to recruit the fibres according to their size.

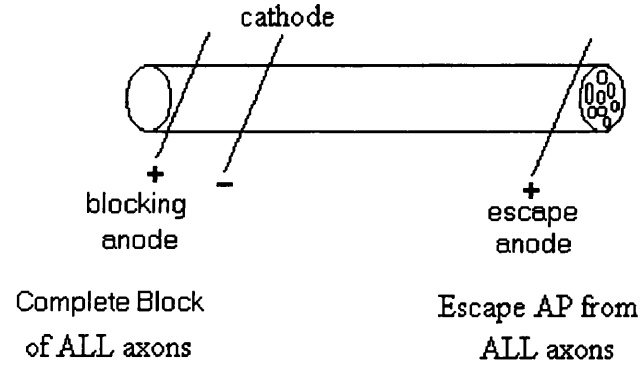
A special tripolar stimulation strategy was adapted to find the optimum parameters for recruitment and uni-directional propagation of action potentials in myelinated fibres.

A cuff tripolar electrode was modelled with internal diameter of 2mm so as to accommodate nerve trunk diameters of about 1.8 mm, this been assumed as the typical diameter of the sural, the tibial or the sacral roots of an adult rabbit. The conductivity of the epineurium of these nerves was assumed to be isotropic and to have conductivity higher than fat ($0.04Sm^{-1}$). Weerasuriya et al (1984) measured the resistance of the perineurium of the frog sciatic nerve as $0.048\Omega m^{-1}$. The conductivity of the nerve modelled was calculated as $2x10^{-4}Sm^{-1}$ assuming a perineural sheet 10μm thick. The range of myelinated nerve fibre diameters used in the model (4 to 12μm) was based on information of Rexed (1944) and Quilliam (1956). The larger nerve fibres (10 to 12μm) were modelled as having internodal length of 1mm, after Ferguson et al (1987).

Two conductivity compartmental models were used. The first excludes the perineurium, simplifying the model and reducing the stimulation run times. The second was based in the results of the first model and included the perineurium. A simple non-fascicular nerve trunk was modelled with the centre node of the fibre placed as to leave a node of Ranvier directly under the cathode.

Model Predictions:

A - First prediction:



B - Second Prediction

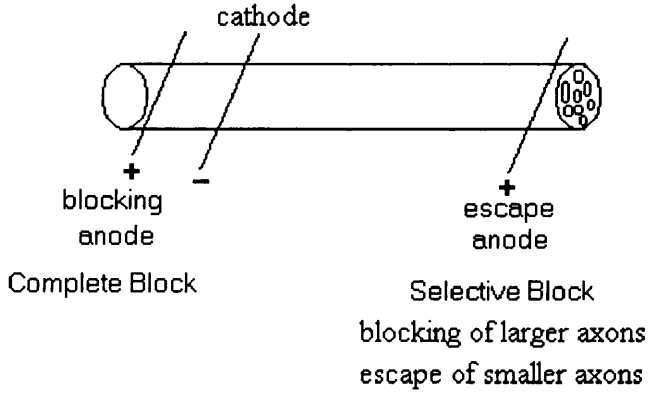


Figure 7

A - The cuff electrode should be able to produce a total anodal block at one end of the cuff and generate unidirectional action potentials at the escape anode.

B - The other aim of the model is to block all the axons at the blocking anode but provide at the escape anode a selective blocking, i.e., an anodal block of the larger diameter axons whilst allowing the propagation of action potentials by the small diameter axons.

Initially three cuff lengths were calculated, and the cathode position was tested at 20% and 30% along the cuff length from the closest anode. The simulation current pulses were rectangular and monophasic having pulse durations of 300 and 500 μs. The ratio of anodal currents was varied between 1:9 and 9:1. The best recruitment sequence was found, for each cuff tested, with an anodal ratio 0f 9:1 (blocking anode: escape anode). The results also favour a cuff position of 20% along the cuff length for cuffs of 6 to 10mm.

Fitzpatrick model used observations of Ferguson et al (1987) who modelled nerve fibres with internodal distances between 1mm and 2mm for nerve fibre diameters of 10μm and 20μm respectively. According to the position of the cathode, the inter-electrode distance between the distal anode and the cathode can be the same as the inter-nodal distance in the larger axons. Although a cuff of 6mm also showed the successive blocking of fibres with an increase in current, with the cathode ideally placed at 20%, the inter-electrode distance between the blocking anode and the cathode will be 1.2mm. This corresponds to a fibre diameter of 12μm. Therefore, the internodal distance in fibres larger than 12μm would be greater than the inter-electrode distance of this particular size of cuff. Increasing the cuff length will also increase the inter-electrode distance between the distal anode and the cathode.

The model investigated the threshold current dependence for different electrode contact widths and nodal positions under the cathode. The initial model placed a node of Ranvier directly under the cathode. Then, the nodal position was varied by up to half its axial internodal length, on either side of the cathode. The variation in internodal distance in a range of axons of different diameters may cause the threshold currents to be higher than expected in the model.

The evaluation of fibre recruitment studied in the model showed that successive blocking of the larger fibres ought to be achieved with an increase in the stimulation current. The action potential and the unidirectional action potential thresholds were different for each cuff tested, this variation being related to the inter-electrode distance. However, there was no significant difference in the respective thresholds by using pulse durations of 300 and 500 μs although the blocking threshold was higher with 200 and 300 μs compared with 500 μs. The model predicts that an anodal current ratio of 9:1 could produce unidirectional action potentials in all axons, each successive smaller fibre being blocked with an increase in current. The smaller axons showed a greater dependency on the radial distance from the cathode.

The external medium surrounding the nerve bundle has been assumed to be isotropic and homogeneous and has been modelled as having conductive properties similar to fat. However, in animal models it is expected that other tissues such as muscles and blood may disturb the external electrical field. The model also does not take into account the presence of other nerve axons nearby because only one nerve axon is modelled at a time. However, in life there is more than one nerve fibre per nerve trunk and thus the available current for each fibre will decrease. Also, the myelin sheath surrounding the nerve axon was modelled as a perfect insulator. It is expected, therefore, that the actual stimulation currents would be higher than those predicted in the model.

Table 3 summarises the effects of a range of currents applied to axons between 4 μm and 12 μm in diameter within a cuff electrode. The currents tested varied between 325 μA and 800 μA. The duration of stimulation pulse was 300 μs. The calculations were performed for anodal currents split in various ratios: 7:3, 8:2, 9:1. The most important predictions are the ones that show block of action potentials in the

largest axons with selective activation of the small axons. They appear in the shaded boxes in the table. For example, during stimulation with 800 μA for an anodal ratio of 9:1, axons with 12μm, 10 μm and 8 μm diameters were blocked but action potentials were still propagated in the 6 μm and 4 μm diameter axons.

Conclusions from the model studies according to Fitzpatrick:

Unidirectional action potentials were fired at the farthest anode in all cuff lengths tested with the cathode positioned at 20% and for an anodal ratio of 9:1.

There were no significant differences between the magnitude of current thresholds for generation of unidirectional action potentials. There was, however, a difference in the blocking threshold currents.

The best recruitment order of the fibres was achieved with the cathode at 20% and for an anodal ratio of 9:1.

The smaller fibres showed a greater dependency on the anodal ratios and on their radial distance from the cathode. The greater the anodal ratio, the greater the threshold for the smaller fibres. The same is true in relation to the radial distance from the cathodal.

The nerve trunk was modelled as non-fascicular. However, if perineurium is introduced into the compartmental model, a greater current is needed to produce unidirectional action potentials and the blocking action becomes less selective.

Table 3 - Predictions of the effects of a range of currents applied to axons of diameters between 4 μm and 12μm in a cuff electrode (Fitzpatrick, 1994).

This table shows the recruitment order and the propagation and blocking threshold currents of action potentials in different axon diameters at different anodal stimulation currents. The ideal response is action potentials travelling in the larger fibres being blocked at both ends of the cuff (X) whilst action potential in the smaller fibres propagate past the escape end of the cuff (→).

Anodal ratios of 7:3 - A total block occurred at both ends of the cuff in larger axons (10 and 12μm) with all the currents intensities tested. Currents intensities between 325μA and 451μA produce an anodal block of all larger axons at both ends of the cuff but allow propagation of action potentials in 8μm axons. However, unidirectional action potentials are achieved with current ranges from 325μA to 600μA in 6μm axons and above 458μA in the 4μm axons. Also, propagation at both ends of the cuff occurred in the smaller fibres.

Anodal ratios of 8:2 - Action potentials propagating in the larger axons are blocked with current intensities above 371μA. Action potentials in the 6 and 8μm axons escape at one end of the cuff with current intensities between 371μA and 555μA. Still some propagation in both ends of the cuff is seen in smaller fibres at lower current intensities. **Anodal ratios of 9:1** - Action potentials propagating in the larger axons are blocked with current intensities above 451μA. All the action potentials in smaller axons (4 and 6μm) propagate at the escape end of the cuff. No bilateral propagation is seen.

Anodal current ratio	Size of axon (μm)	Current (μA)										
		325	371	410	428	451	454	458	555	600	636	800
7:3	12	x	x	x	x	x	x	x	x	x	x	x
	10	x	x	x	x	x	x	x	x	x	x	x
	8	→	→	→	→	→	x	x	x	x	x	x
	6	→	→	→	→	→	→	→	→	→	x	x
	4	↔	↔	↔	↔	↔	↔	→	→	→	→	→
8:2	12	x	x	x	x	x	x	x	x	x	x	x
	10	→	x	x	x	x	x	x	x	x	x	x
	8	→	→	→	→	→	→	→	→	x	x	x
	6	→	→	→	→	→	→	→	→	→	x	x
	4	↔	↔	→	→	→	→	→	→	→	→	→
9:1	12	→	→	→	→	x	x	x	x	x	x	x
	10	→	→	→	→	→	→	→	x	x	x	x
	8	→	→	→	→	→	→	→	→	→	→	x
	6	→	→	→	→	→	→	→	→	→	→	→
	4	→	→	→	→	→	→	→	→	→	→	→

Key: ↔ action potential propagating from both ends of cuff
→ action potential propagating from escape end of cuff
x anodal block of action potentials at both ends of cuff

Modelling s predictions:

1- It may be possible to generate and propagate action potentials which are anodally blocked at one end of the cuff but which are allowed to escape from the other end.

2 - It maybe possible to regulate the intensity of the anodal current at the escape end of the cuff to provide for selective blocking of the large diameter axons whilst allowing for the escape of action potentials in the smaller axons (Fitzpatrick et al, 1994).

These predictions are in general agreement with similar independent studies (Rijkhoff, 1994).

These studies have been most encouraging. However it is essential to test the electrode behaviour by stimulating nerve axons of alive tissues. Therefore, a tripolar electrode was constructed based in the ideal parameters predicted in the model and a series of experiments was designed to test the properties of unidirectional excitation and reverse recruitment sequence.

This research began with the tests of this electrode firstly in a peripheral nerve preparation, the sciatic and the tibial nerve of rabbits. The ideal parameters for stimulation and blocking were observed and improved for use in an animal model. Then, the possibility of selectively activate the bladder by stimulating the sacral root using this tripolar stimulation strategy was evaluated in rabbits.

Objectives

1 - To test the feasibility of the model predictions in an animal model. To evaluate the tripolar cuff electrode and the stimulator proposed by Fitzpatrick.

2 - To test if it is possible to selectively activate the bladder without concurrent contraction of the urethral external sphincter by using sacral root stimulation.

3 - To test if it is possible to achieve an anodal block of the larger somatic motor axons.

4 - To test the ideal stimulus parameters to achieve selectivity.

CHAPTER FIVE
PRELIMINARY TEST OF THE TRIPOLAR CUFF ELECTRODE

Chapter 5 - Preliminary test of the tripolar cuff electrode

A series of experiments was designed to test the modelling predictions.

A tripolar electrode and a stimulator were constructed and experiments were carried out in sciatic and tibial nerves of New Zealand white rabbits. These peripheral nerve preparations were used to test the electrode and the ideal parameters for stimulation and blocking of axons.

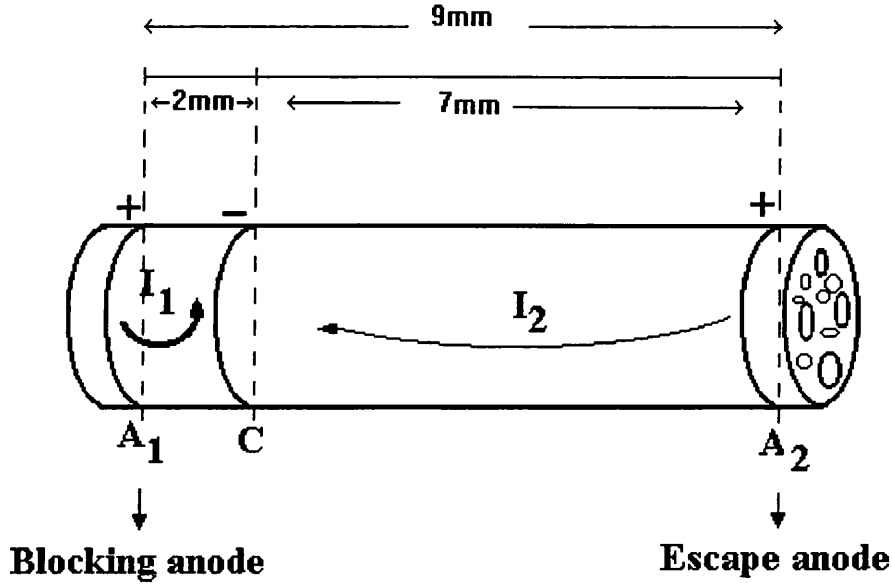
5.1 - Electrode construction

Details of the electrode construction are published as part of the PhD thesis of Fitzpatrick (1994). Brief details are given here to aid the interpretation of data.

The electrode has three platinum ring electrodes. Each ring electrode was formed by wrapping a 0.5 mm high purity platinum wire (Goodfellow Ltd) around a steel rod. The diameter of the steel rod determines the internal diameter of the cuff. The three ring electrodes were placed onto a polypropylene spindle with two silicone tubing spacers between the electrodes so that the distance between them was kept as required. The electrodes were cover by silicone tube.

The more centrally located ring represents the cathode, which is asymmetrically placed in between two anodes. The electrode measures 9 mm in total. The distance between the first anode and the cathode is 2 mm, and the distance between the second anode and the cathode is 7 mm. The first anode will be referred to in the future sections as the blocking anode. The second anode will be referred to as the escape anode. The diagram in figure 8 shows the electrode design.

The Tripolar Cuff Electrode



$$I_1 + I_2 = \text{Cathodal Current}$$

$$I_1 > I_2$$

Figure 8 - Tripolar electrode design based on the Fitzpatrick Model. The electrode has three platinum ring electrodes placed in a propylene tube. The cathode (C) is asymmetrically placed in between two anodes (A_1 and A_2). The electrode length is 9 mm. The distance between the first anode (A_1) and the cathode is 2 mm and the second anode (A_2) and the cathode is 7 mm.

All the specifications of the electrodes were based on the predictions of the modelling study, already presented in chapter 4. Two current sources are required to deliver the anodal currents which sum together to form the cathodal current. A two channel constant current stimulator allowed switching between fixed ratios of anodal currents, e.g. 9:1, 8:2, 7:3. These ratios describe how the cathodal current is divided to produce the anodal currents. The total current (cathodal current) may vary from zero to the maximum current allowed by the stimulator which is 5 mA.

It is also possible to use this stimulator in bipolar mode. During bipolar stimulation the two outer electrodes are used.

During the experiments, trigger pulses, which set the duration of the stimulus current, were generated with a Neurolog NL 300 pulse generator, a Neurolog NL 403 delay-width and a Neurolog NL 510 pulse buffer. The intensity of stimulating currents was measured by observing the voltage drop across a 50 Ohm resistor in series with each anode. Either anodal current could be displayed on an oscilloscope 1. Details of the stimulation applied during the experiments will be discussed later in this chapter.

5.2 - Electrode structure: silver or platinum wires?

Stimulating electrodes which are placed upon nerves are important components of many implantable neuroprothesis. However, the electrode structure and the components used during the electrode construction may interfere with the final result of stimulation. Several reports on electrode material and chemically reversible charge injection protocols have appeared in the last 40 years (Brummer et al, 1983; Dymond, 1976; Greatbatch and Chardack, 1968; Loeb et al, 1982; Piersma and Greatbatch, 1987; Weinman, 1965).

There are two electrode materials commonly used in experiments. Silver electrodes have been used for a long time. It is cheaper and responds satisfactorily in the majority of acute experiments. However, it is liable to polarise during use and this may interfere with the flow of ionic charges in biological tissues. Platinum electrodes are much less like to polarise, but they are much more expensive.

A simple experiment was designed to test which material would be more appropriate to use in these experiments.

The resistance of a silver cuff electrode and a platinum cuff electrode were measured in 3 different experiments. Measurements of electrode resistance were made as current was passed through: an empty electrode in a saline solution and an electrode containing a dead sciatic rabbit nerve or a piece of thread. The presence of the dead nerve reduced the volume of saline in the cuff. This was an attempt to reproduce experimental conditions more faithfully. During the stimulation, the electrical current was kept constant (1mA) and the frequency varied from 10 Hz to 200 Hz. The first three measurements were made at 10 Hz, at intervals of 5 minutes between each one of them. Successive measurements were made at 20Hz, 50Hz, 100Hz and 200Hz, thus measurements of

resistance were made at 20 Hz, at 15, 20 and 25 minutes; 50 Hz at 30, 35 and 40 minutes; 100 Hz at 45, 50 and 55 minutes and 200 Hz, at 60, 65 and 70 minutes. The results are plotted in figure 9. This compares the changes of resistance observed for the silver and platinum electrodes as a function of time and frequency of stimulation. The graph shows that the resistance of the silver electrode increased progressively. After 70 minutes the resistance was almost twice the initial values. These phenomena strongly suggest electrode polarisation.

However, there was no great increase in resistance of the platinum electrode. This suggests that polarisation was not a problem and all future experiments were done using platinum electrodes.

Figure 10 plots the resistance measured through a silver (A) and a platinum (B) electrode against the logarithm of the number of pulses applied during stimulation. The resistance of the silver electrode increased proportionally with the number of pulses until around 160.000 pulses. After this, the resistance was stable. The silver electrode offers greater resistance at the end of stimulation, so may interfere with the final results. In contrast, the resistance of the platinum electrode is more stable.

These experiments confirm that polarisation is more likely to occur with silver electrodes and it may interfere with the results of stimulation. Therefore, the platinum cuff electrode was chosen to be used during this research.

Comparison of variations in resistance in silver and platinum electrode according with the frequency of stimulation and time

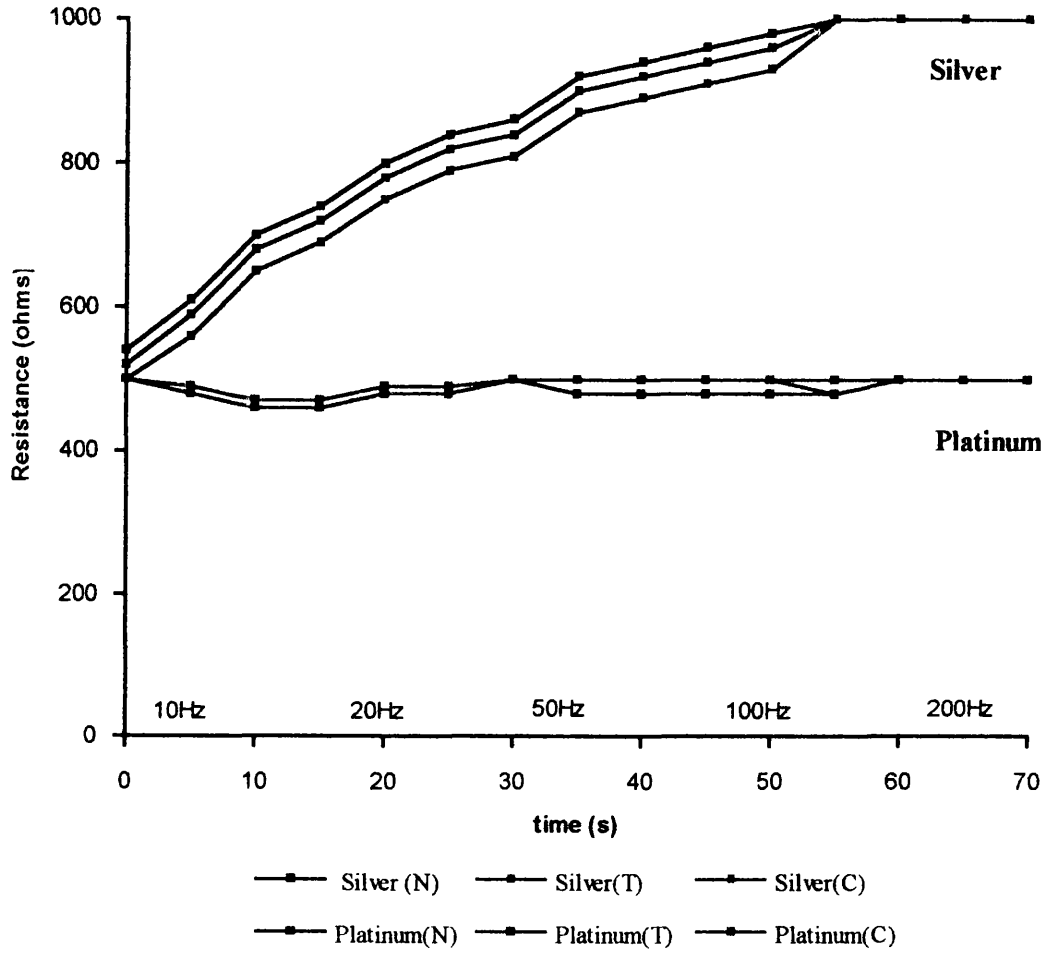
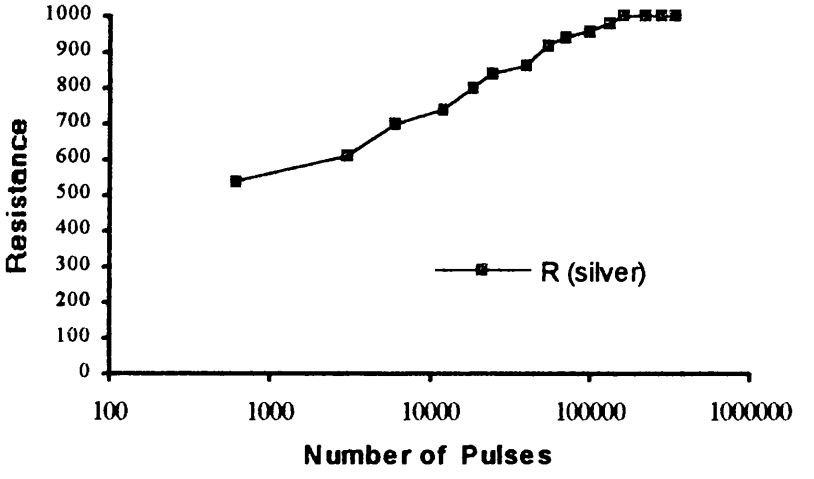


Figure 9 - Changes in the resistance of silver and a platinum cuff electrode when constant currents pulses are delivered at a range of frequencies. The cuff were immersed in physiological saline. They were tested in three conditions: empty cuff (C), containing a sciatic nerve (N) or thread (T).

The frequency of stimulation was increased at intervals through the test as shown. Initially, the silver and platinum electrodes had similar resiatances but whilst the resistance of the platinum electrode was constant the silver electrode polarised rapidly.

Comparison of variations of resistance in silver and platinum electrode with increasing number of stimulation pulses.

A - Silver electrode



B - Platinum electrode

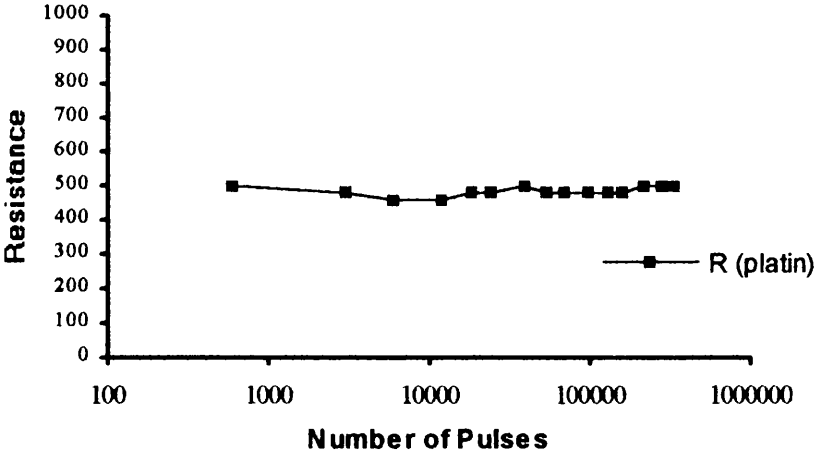


Figure 10 - Resistance of a silver cuff electrode and a platinum cuff electrode as a function of the number of stimulation pulses delivered. The resistance of the silver electrode increases progressively.

5.3 - Is the rabbit suitable for these experiments?

The mathematical model developed by Fitzpatrick predicted the responses of myelinated axons in the tripolar cuff electrode. He modelled the effects of currents applied to axons among 4 and 12 μm.

Histological studies of rabbit sciatic and tibial nerves were performed to establish if their range of axon diameters was within the range studied in the model.

5.3.1 - Histological methods:

Light Microscopy

Sections of sciatic and tibial nerve dissected from rabbit were fixed in Carson's phosphate buffered formalin (Carson et al, 1973), dehydrated in graded alcohol and embedded in Paraplast. Serial sections of 10μm thickness were cut using a Leica 2050 microtome. The sections were then stained with phosphotungstic acid haematoxylin (P.T.A.H.) or Miller's elastic stain (Miller, 1971) and Van Geison (1889). The stained sections were examined using a Zeiss Axiophot microscope and photomicrographs taken on Kodak Ektachrome 64 film.

Electron Microscopy

Sections of sciatic and tibial nerve dissected from rabbit were fixed for 1 hour using 2% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2 (Sabatini et al, 1963). The specimens were then given three 20 minute washes in 0.1M sodium cacodylate buffer at pH 7·2. Post-fixation was carried out using 1% osmium tetroxide in 0.1M sodium cacodylate buffer at

pH 7.2. Following fixation the specimens were dehydrated with graded alcohol and embedded in Araldite (Glauert, 1991).

Ultrathin sections were cut with a Diatome diamond knife on an L.K.B. 3 Ultrotome and mounted on Formvar coated 1000μm aperture grids. The ultrathin sections were double stained with uranyl acetate (Stempak & Ward, 1964) and lead citrate (Reynolds, 1963).

The stained sections were examined using a Zeiss E.M.109 electron microscope at 50kV and electronmicrographs taken on Ilford FP4 film.

Photographic prints were made from the best sections of the tibial nerve. The total nerve fibre was reconstructed in a photomontage from where the total diameter of each axon was measured.

5.3.2: Results:

The photomontage of a transverse section of a rabbit tibial nerve is presented in figure 11. The larger, thickly myelinated axons can easily be seen. However, smaller more thinly myelinated axons were also seen as were many unmyelinated axons. A more detailed picture is presented in figure 12. Sometimes it is easy to observe a clear division into a group of larger axons and a group of smaller axons. According to Boyd (1968), the thickness of the myelin sheath can vary and it is not always proportional to the axon size. This effect is greater in smaller axons. So, even smaller axons can also be divided in those which are thickly myelinated and those which are thinly myelinated. These observations can be made in figure 12.

All axons were counted and their diameters calculated and compared with the range studied in Fitzpatrick model.

Axons diameter were obtained in two ways. Axons shrink and change their shapes after fixation. Therefore, some of the axons lost their

circular shapes. In non circular axons, the measurements was taken at the largest point of the axon, and it included the sheet of myelin. Once more, Boyd studies (1968) on composition of peripheral nerve were used as a guide. Axons were also measured by using a map measurement device which allows more accuracy. This method gives the total circumference of the axon, independent of its shape. The diameters of these axons were then calculated by using the formulas of circumference and diameter.

$C = 2\pi R$ and $D = 2R$

so, $C = D\pi$

C = circumference

π = constant (3,1417)

R = radius

D = diameter

The results obtained by measuring the diameter of axons were compared with the results obtained by calculating the diameter from circumference obtained by using the map measurement device and they were similar.

The thickness of the sheet of myelin was also measured. The measurement was taken at the same point where the total axon diameter was measured.

The distribution of axon diameters is presented in a fibre-size histogram in figure 13. As it was expected, the range studied in the model is well represented in the rabbit peripheral nerve. Thus, rabbit nerve can be concluded to be suitable for these experiments.

Frequency histogram of the tibial nerve axons

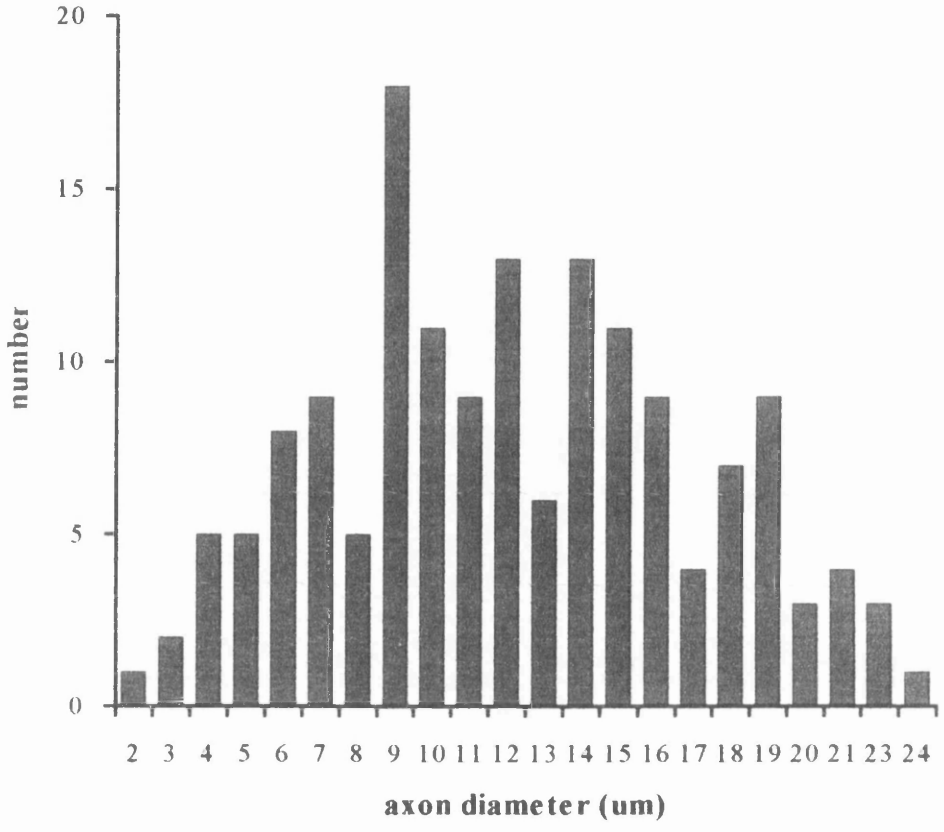


Figure 13 - Fibre diameter histogram showing the distribution of axon diameters in a section of the tibial nerve. This study was made to confirm if the range of axons diameters in the mathematical model was present in the nerve fibre. It can be concluded from the histogram that the range of axon diameters studied in the model (4μm to 12μm) are well represented in the rabbit tibial nerve.

5.4 - Testing for unidirectional excitation

The first prediction of the modelling studies is that it may be possible to generate and propagate action potentials which are anodally blocked at one end of the cuff but are allowed to escape from the other end.

This prediction was tested in 14 experiments on isolated nerve trunks of New Zealand rabbits. These *in vitro* peripheral nerve preparations were useful to test the electrode and to try to set up the ideal parameters for stimulation and blocking of axons before starting the *in vivo* experimental models.

5.4.1: Methods

Sciatic and tibial nerves were dissected from 14 recently sacrificed adults New Zealand white rabbits. Time between death and mounting the nerve in the bath was no longer than 15 minutes.

A latero-posterior incision in the hindlimb, from the gluteus down to the rabbit's ankle joint allowed complete exposure of the nerve trunks. The gluteus maximus muscle was reflected to exposed the great sciatic nerve. Continuing the dissection down until the calcaneal tendon allowed dissection of the sciatic together with the tibial nerve in length that varied between 55 mm to 135 mm. The surrounding fatty tissue was removed. During the dissection, care was taken not to stretch or damage the nerve fibres. After removal, the nerve was held at one end with a linen thread and transferred to a bath of physiological saline warmed to 37°C. The warm saline helped to keep the nerve temperature close to the normal body temperature until the stimulation started.

The nerve was then transferred to a heated chamber containing a bath of light paraffin oil at 37°C. This bath was kept warm during the

experiments by means of thermostatically controlled heating elements built into the operating table. A digital thermometer (Radio Spare Components, UK) was kept in the bath to check the temperature. A bipolar stimulating electrode was placed in the middle of the nerve and two recording electrodes at both extremities. The nerve viability was checked by stimulating it. If the nerve condition was satisfactory, action potentials were recorded at both ends. The nerve was then drawn through a tripolar cuff electrode, which was positioned at the middle of the nerve. The sciatic end of the nerve was placed near the blocking anode, the tibial end of the nerve was placed near the escape anode. Two recording electrodes were located at either end of the nerve trunk. Using only the two outer electrodes of the cuff for bipolar stimulation, the nerve was stimulated using monophasic rectangular pulses. The current was increased until evoked action potentials were detected from both ends of the nerve. Nerves which failed to show action potentials were discarded. The stimulator was then, switched to tripolar mode.

During tripolar stimulation, the ratio of anodal currents were varied in attempts to generate an anodal block at one end of the cuff electrode. The anodal block was identified by the absence of action potential near the blocking anode. Once an anodal blocking have been set up, the stimulus parameters were changed to test for other possible combinations. In addition to the anodal ratios, a range of pulse durations was tested, from 60 μs to 1000μs.

Figure 14 represents the experimental set-up. Figure 15 shows the stimulator and the cuff electrode constructed to be tested during the experiments.

Experimental Set-up

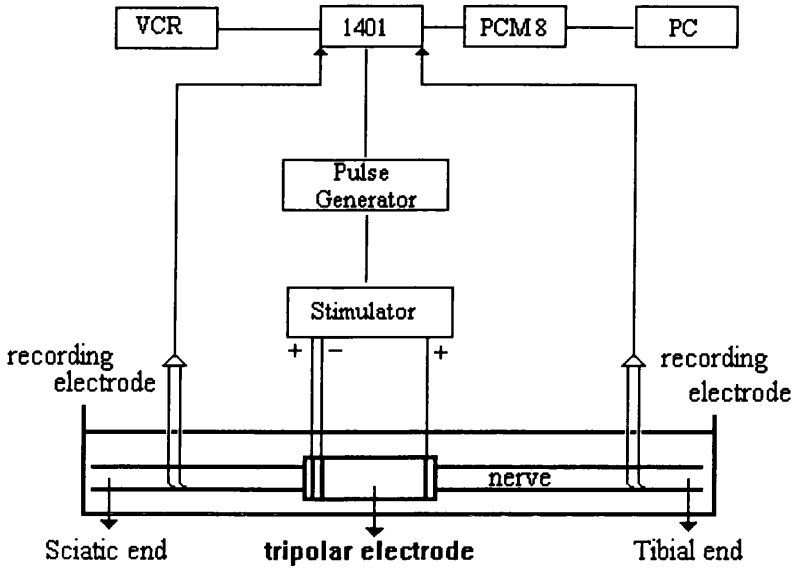


Figure 14 - Experimental set-up to test the unidirectional excitation properties of the tripolar cuff electrode. A nerve trunk was positioned in a paraffin bath. A tripolar cuff electrode was slipped onto the nerve. The sciatic end of the nerve trunk was positioned near the blocking anode. The tibial end was positioned near the escape anode. Two recording electrodes were placed at each end of the nerve trunk. The condition of the nerve was checked by stimulating the nerve by means of the cuff electrode and recording at both ends. The responses were signal averaged on line via a peripheral interface (1401). The data was also recorded onto a video tape (VCR) via an A/D multiplexer (PCM-8).

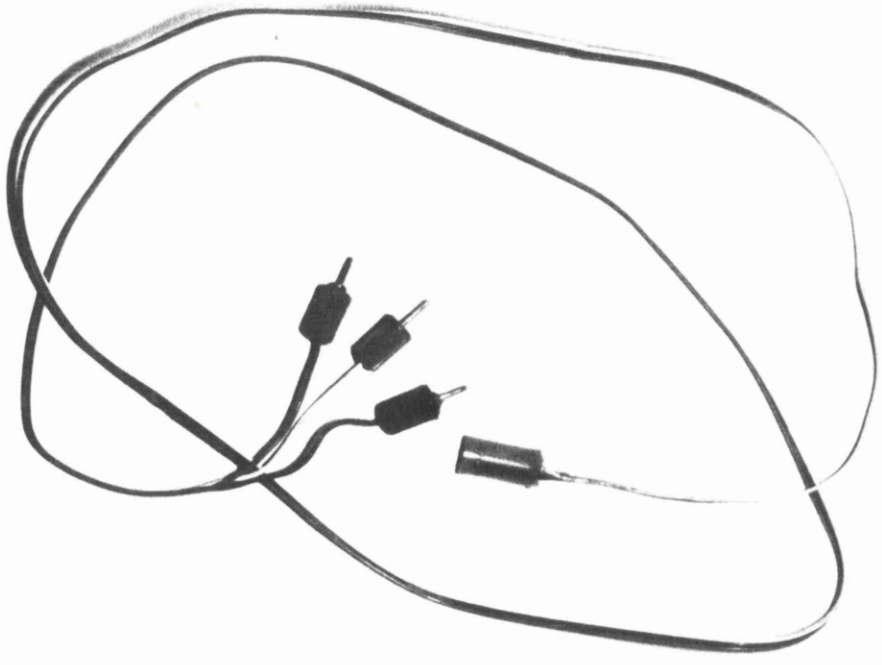
Figure 15 - Fitzpatrick Stimulator (Mark VI) - The two channel stimulator can be used either to deliver bipolar or tripolar stimulation. The total current delivered can be controlled by means of the source current switch and the current range switch. These allow the current range to vary from 0 to a maximum of 5mA. During tripolar stimulation a current ratio can be set (current ratio a1:a2 switch).

The cuff electrode connects to the red (a1 and a2) and black (c) sockets at the right-hand side of the stimulator as shown in the photograph. Details of the structure of the cuff electrode was already described in figure 8, pg 45.

Fitzpatrick stimulator and tripolar cuff electrode

Stimulator:

Tripolar Cuff Electrode



5.4.2: Results

The first prediction of the modelling studies is that it may be possible to generate and propagate action potentials which are anodally blocked at one end of the cuff but are allowed to escape from the other end. The rabbit sciatic nerve was stimulated by means of Fitzpatrick cuff electrode and the response was recorded at each nerve end. During tripolar stimulation, compound action potentials were observed at one end of the nerve but no action potential occurred at the other end in 11 out of 14 experiments. In the remaining three experiments action potentials were fired at both ends of the nerve during bipolar and tripolar stimulation and block was not achieved.

Figure 16 shows compound action potentials recorded in one of the successful 11 experiments. During bipolar stimulation, action potentials are fired from both ends of the cuff electrode. These action potentials are represented in traces *a* and *b*. However, on switching the stimulation to tripolar mode, no action potential appears at the blocking end (trace c).

The compound action potentials in figure 16 are probably due to activation of axons with conduction velocities faster than 30m/s. This value was calculated based on the time taken until action potential arrived and the distance between the cathode and the recording electrodes. In this experiment, the distances between cathode and recording electrodes are 22 mm at the blocking end and 16 mm at the escape end. It is very difficult to calculate the latency period in figure 16 **a** and **c**, due to the big stimulus artefacts. In turn, the time between stimulation and the action potential peak was calculated as 0.8 ms, so overall conduction velocity of axons activated is 27m/s. At the action potentials at the escape end, at figure 16 **b** and **d**, the latency period is more easily observed. There are smaller stimulus artefacts

at these points, because the current delivered is smaller. The conduction velocity of the faster fibres at the escape anode is about 30 m/s.

The expected conduction velocities in the larger axons are about 90m/s. However, the compound action potentials in figure 16 are probably due to activation of axons with conduction velocities around 30m/s. This difference is probably due to changes in the nerve condition due to experiment delay. Stimulation began at least 30 minutes after the nerve have been removed from the rabbit. In addition, some of the experiments took as long as two hours to complete. Although action potentials were still recorded in such experiments, the nerve condition may not be the same throughout the end of the experiment. This will be discussed in more details later in this thesis.

Action potentials from substantially smaller, slower fibres are not seen in figure 16. There are several possibilities which might explain this:
- the distance between recording and stimulating electrodes may be too short to allow clear separation of faster and slower components of the volleys.
- the stimulus may be not strong enough to excite the smaller fibres,
- the compound action potentials may be too small to be recorded with this recording electrode configuration at relatively low gain.

The action potential appearance is different at the two ends probably because of different ratios of active/inactive axons. The differences in distance from the cathode to the recording sites plus the small difference between pairs of recording may also interfere in the appearance of action potentials, but these are probably insignificant. Thus, comparison between compound action potentials should be done by comparing the results at one end (bipolar/tripolar) rather than between the blocking and the escape ends.

Compound action potential recorded at the cuff electrode

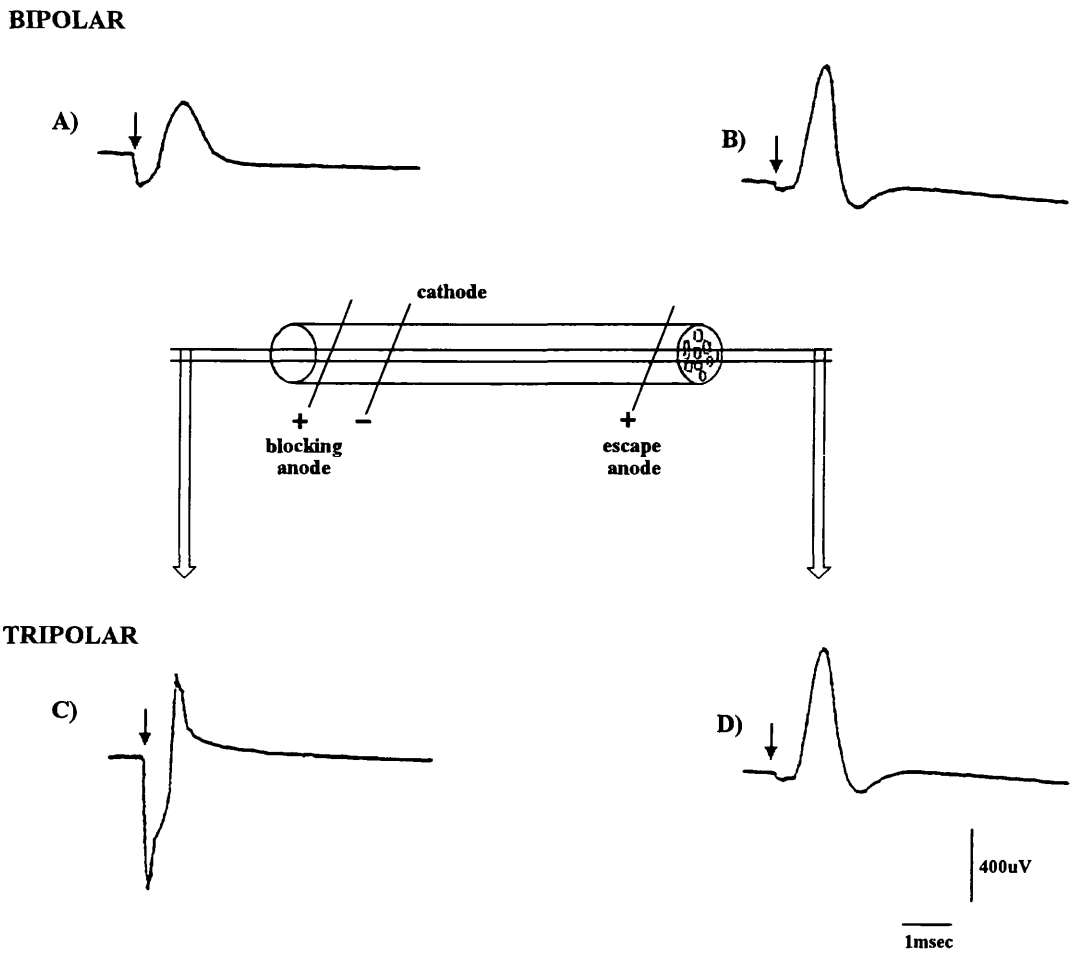


Figure 16 - Compound action potential recorded at both ends of the tripolar cuff electrode during stimulation of a sciatic nerve. A and C are recorded on the same side as the blocking anode. B and D are recorded at the same side as the escape anode. During bipolar stimulation compound action are fired at both ends of the cuff, confirming the two ways of propagation of action potentials. However, during tripolar stimulation a complete blocking occurs at the blocking anode (C) whilst an escape action potential is still recorded at the escape anode (D).

Arrows show when the stimulus is delivered. The stimulus duration is 300μs. The bipolar current is 92μA. The tripolar currents are 92μA and 24μA.

The artefacts also look different. This is probably due to much more current being delivered to the blocking anode during tripolar stimulation.

Artefacts obscure the earliest part of the action potentials in some cases making latency measurements difficult for the very fast fibres. Peak negativity is easier to establish in these cases.

Several factors may influence the achievement of unidirectional blocking such as variation in the pulse duration and the ratio of anodal currents. The modelling studies offer a series of combinations. These combinations are presented in table 3 at chapter 4. As many as 165 options can be modelled. However, not all the combinations were tried during this research.

Anodal block was achieved with 19 different combination during 14 experiments with the sciatic and tibial nerve. The smallest blocking currents were seem with a pulse duration of 400μs. However, blocking was achieved with 300μs in 9 experiments. Although there are variations in the anodal currents, an anodal ratio of 9:1 produced a block in 8 experiments.

Each of the stimulation parameters will be studied in turn.

The model predictions for achieving blocking and the experimental results.

The design of the Fitzpatrick tripolar electrode relies on the relative distance between the cathode and the anodes and on the ratio of the anodal currents. The intensity of current flow in the electrode is a major determinant of the behaviour of the axons. Firstly, there must be sufficient current to excite at the cathode. Secondly, the intensity of current at the anodes must allow for a blocking action.

The aim of the cuff is provide a strong anodal block at one end and a weaker or selective block at the other end. Consequently, independent control of the current at each anode was required. The magnitude of the currents could be controlled independently and they added to provide the cathodal current.

Table 4 shows the experimental results obtained by stimulation of sciatic nerve. The model predicts that blocking is better achieved with stimulation periods of 300μs, so the experimental results obtained at this duration are compared with the modelled results.

Firstly, the cathodal current was analysed to see if there is sufficient current to excite at the cathode. Then, the anodal currents to achieved blocking were compared and finally, the effects of pulse duration in the experimental results are analysed according to the model predictions.

Table 4 - Experimental results showing the 19 combinations of pulse width, current and current ratios which produced an anodal block at the blocking end of the cuff electrode during stimulation of the sciatic nerve. The model predicts block with 300μs and 500μs. The highlighted area represent the range of experiments which may satisfy the model conditions.

The results are sorted by the pulse durations used. The currents are expressed in mA. The anodal ratios are presented in two ways:

- by the theoretical ratio in which the cathodal current is divided to provide the anodal currents as described in the model. This ratio is determined by the current ratio switch at Fitzpatrick stimulator and it is referred to as the anodal ratios (block: esc) in the table.
- by the ratio measured during the experiments. This ratio is calculated by dividing the blocking current($I_{b)}$ by the escape current ($I_{e)}$ as measured during the experiments (in the table, Anodal ratio I_b/I_e).

Pulse width (μs)	Cathodal current (mA)	Anodal ratios (Block : Esc)	Anodal ratios (I_b/I_e)	Current at blocking end (I_b) mA	Current at escape end (I_e) mA
60	1.28	0.6:0.4	1.5	0.76	0.52
60	1.68	0.7:0.3	2.2	1.16	0.52
60	1.72	0.7:0.3	2.3	1.2	0.52
100	1.5	0.5:0.5	1.1	0.8	0.7
100	3.4	0.8: 0.2	4.7	2.8	0.6
100	1.32	0.9:0.1	10	1.2	0.12
200	1.5	0.5:0.5	1.1	0.8	0.7
300	1.04	0.5:0.5	1	0.52	0.52
300	1.24	0.5: 0.5	1.2	0.68	0.56
300	0.276	0.8:0.2	4.3	0.224	0.052
300	1.32	0.8:0.2	5.6	1.12	0.2
300	0.68	0.9:0.1	7.5	0.6	0.08
300	1.4	0.9:0.1	11	1.28	0.12
300	1.52	0.9:0.1	12	1.4	0.12
300	1.76	0.9: 0.1	6.3	1.52	0.24
300	2.36	0.9:0.1	18.7	2.24	0.12
400	0.604	0.8:0.2	24.2	0.58	0.024
400	0.65	0.9:0.1	8.3	0.58	0.07
1000	2.16	0.8:0.2	2.9	1.6	0.56

Table 4

The Cathodal current

The intensity of the current which flows at the cathode is an important determinant of the behaviour of the axons because there must be sufficient current to excite an action potential. During the experiments, the minimum current intensity for excitation at the cathode was never tested because the currents were adjusted to produce a block. However, based in the model studies, it is possible to be confident that a great excess of cathodal current was used during the experiments. This is seen in figure 18. Thus, excitation at the cathode should occur in all experiments.

Figure 17 shows the excitation threshold currents for the generation of an action potential for different ratios of anodal currents for each nerve fibre diameter as it is predicted in the model. Less than 200μA was needed to excite the range of axons tested in any anodal ratio. The smaller fibres require a larger threshold current compared to the larger fibres. However, the anodal ratio seems to be less important in these results. This is not surprising since excitation occurs at the cathode.

Figure 18 shows the cathodal currents used during the experiments with the rabbit sciatic nerves, compared to the modelled excitation threshold currents. As the anodal ratio is not important here, and there is almost no variation between the predictions for each anodal ratio, the modelled results for the ratio 9:1 were used as the general model predictions for minimum current to excite. During the experiments, the minimum cathodal current used was 276μA. Therefore, the current used was always greater than the minimum predicted for excitation.

Predicted excitation threshold currents for different fibres sizes

Model predictions

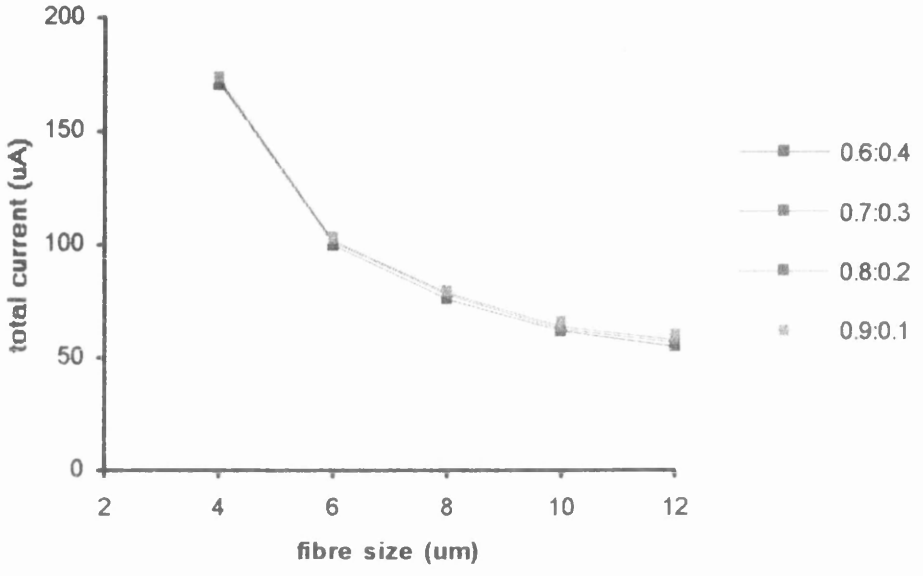


Figure 17 - Excitation threshold current as a function of the fibre diameters at different anodal current ratios as modelled by Fitzpatrick. Pulse duration: 300μs. More current is needed to excite the smaller fibres. However, almost no separation is observed between the thresholds at the various anodal ratios.

Cathodal current used during experiments with the sciatic nerve

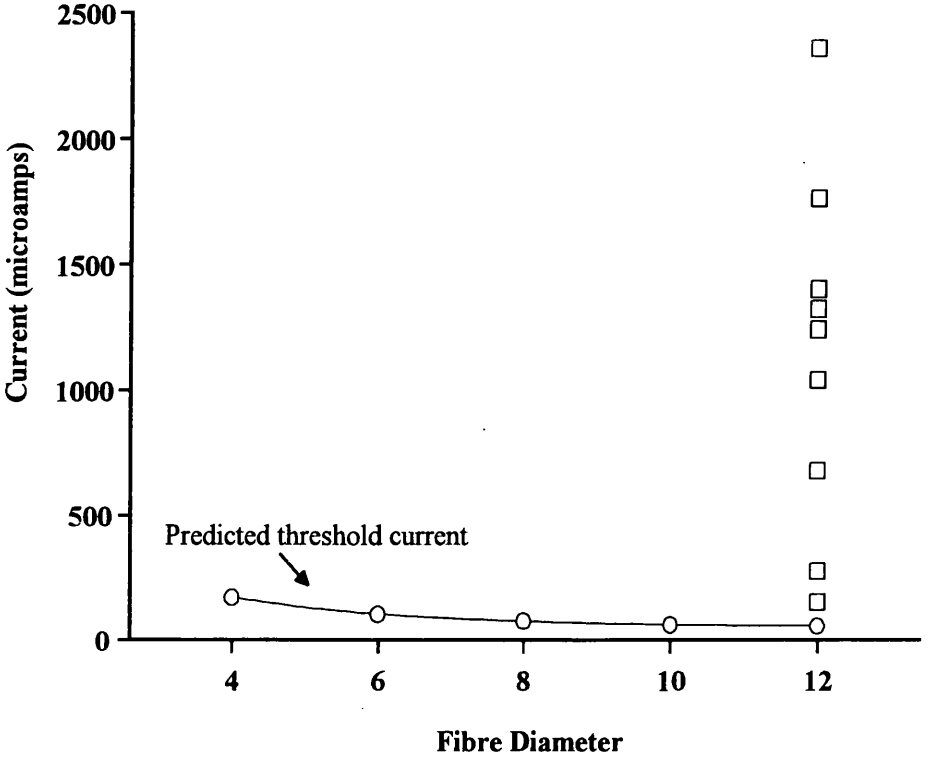


Figure 18 - The cathodal current used during the sciatic nerve experiments are compared to the predicted excitation threshold current. Stimulation pulses last 300μs. All measured cathodal currents are plotted at 12 μm since this is the largest fibre diameter modelled. The current intensities applied during the experiments were well above the predicted excitation threshold.

Key:

0 - model predictions

□ - experimental results

The Anodal currents

Model predictions:

The model calculated the minimum blocking currents as a function of the fibre diameters at different anodal ratios for pulse widths of 300 and 500μs. According to the model, there were no significant differences between the magnitudes of thresholds for these pulse widths. There was, however, a difference in the blocking currents predicted for different anodal ratios These results are presented in figure 19. The coloured curves characterise the minimum stimulus current needed to achieve blocking in four different anodal ratios.

The minimum current for blocking depends on the fibre size and the anodal ratios. The greater the anodal ratio the greater the blocking threshold for all fibres. The predicted minimum blocking current for smaller fibres is greater than that for the larger fibres and is more dependent on the anodal current ratio.

The minimum predicted current intensity for blocking the larger axons is 350μA for anodal ratios of 6:4 and 7:3 at a pulse duration of 300μs. However, greater anodal ratios will require more current to produce a block. The minimum current predicted to block larger axons for an anodal ratio of 9:1 is 600μA.

Predicted minimum blocking currents

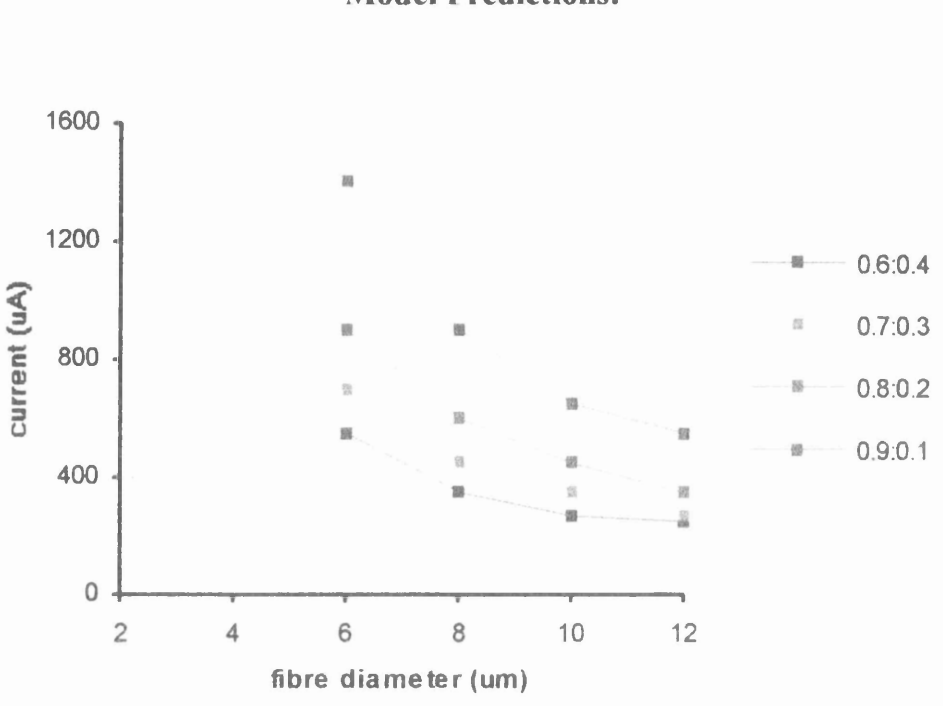


Figure 19 - Minimum blocking currents as a function of the anodal ratio and fibre diameter, as predicted in Fitzpatrick model. More current is needed to block the smaller fibres. Also, the greater separation among the blocking thresholds at 6μm and 8μm axons suggests the smaller fibres are more dependent on the anodal ratio. The greater the anodal ratio, the greater the minimum blocking current.

Experimental Results:

The minimum current required to block the axons during the experiments with the rabbit sciatic and tibial nerves is presented in table 4. The highlighted area represents the range of experiments which worked at 300μs and may satisfy the model. From these experimental results, the minimum current required to produce a block ranged from 224μA to 2240μA. In two experiments the current required for blocking was in excess of the predicted range. In two cases, block was achieved experimentally with less current than predicted. The experimental results confirmed the model predictions that greater anodal ratios required greater current intensities to produce an anodal block. This is illustrated in figure 20, which plots the minimum current needed to achieve blocking in the rabbit sciatic nerve axons as a function of the ratio of anodal currents. Higher anodal ratios are associated with higher blocking current ($p<0.01$).

The predicted blocking currents at each anodal ratio are compared to the experimental results in figure 21 and 22. All measured threshold currents are plotted at 12μm diameter since it is the largest diameter modelled. Generally, more current was needed to produce a block in the axons of the sciatic nerve than was predicted in the model. However, these results can be easily explained since the model calculations fail to include factors as current leaks in to neighbouring tissues and loss of excitability, which may occur in experimental conditions. These will be discussed later in more detail.

Within the experiments performed at a pulse duration of 300μs, no blocking was achieved for an anodal ratio of 6:4 and 7:3. Figure 21 shows the two experiments in which block was achieved for an anodal ratio of 8:2, compared with the modelled results. Assuming that the larger fibres were blocked, in one experiment more current was required to block than predicted in the model, but less current was required in the other.

Minimum blocking current at a range of anodal ratios

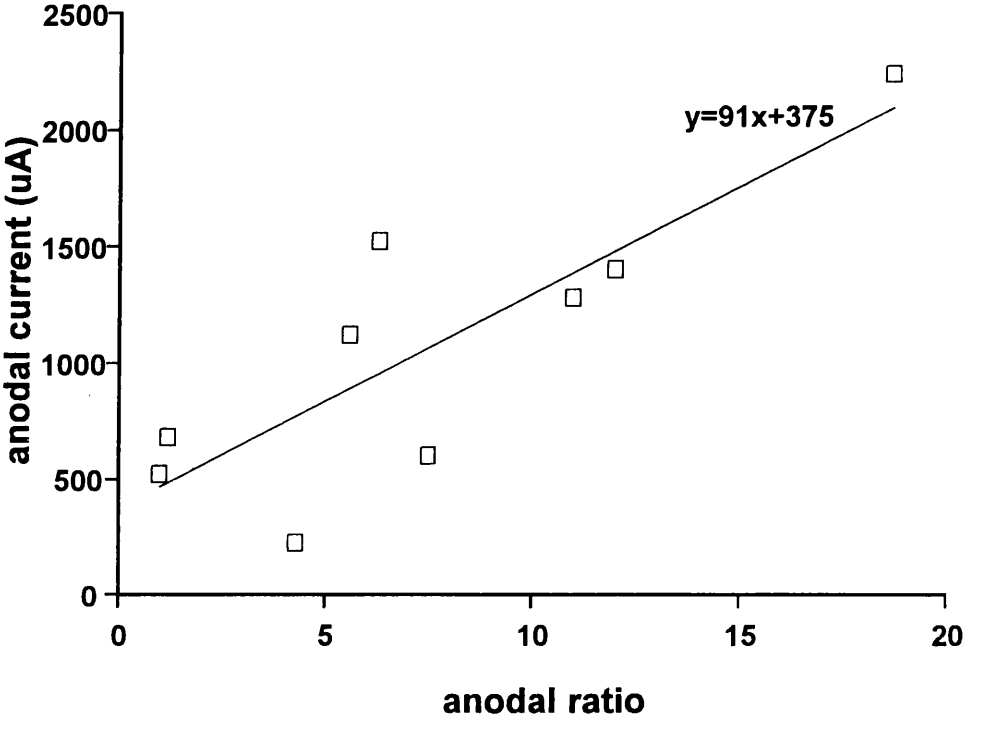


Figure 20 - Experimental data showing the minimum current needed to achieve blocking in sciatic nerve axons for a range of anodal ratios (blocking current/escape current). Greater anodal ratios require more current to block. A linear regression line is fitted to the data. Its formula is $y = 91x+375$ and it has a correlation coefficient of 0.8, $p < 0.01$ (Pearson's linear correlation coeficient).

Minimum current to block axons at an anodal ratio of 8:2

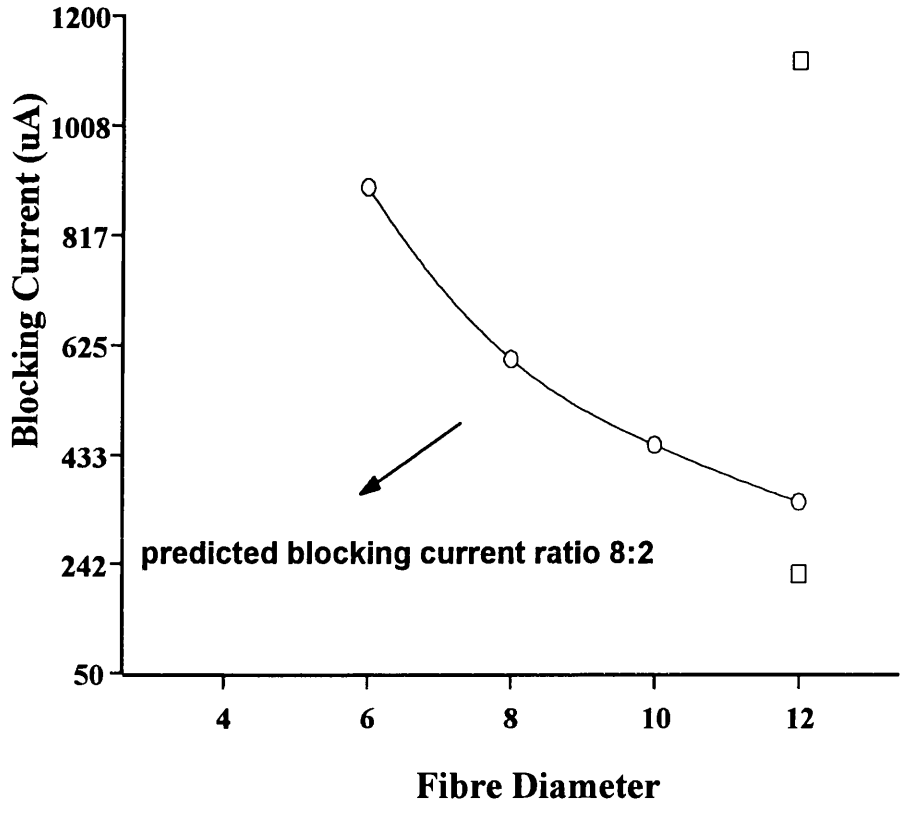


Figure 21 - The minimum anodal current needed to block during stimulation of the sciatic nerve with an anodal ratio of 8:2 (open square) is compared to the minimum currents predicted by the model. Results of two experiments are shown. One exceeds the predicted minimum. The other is below the predicted minimum.

Key:

0 - model predictions

□ - experimental results

The best results were obtained at an anodal ratio of 9:1, which are presented in figure 22. According to the model, the recruitment order table for a pulse width of 300μs (table 3 at Chapter 4) shows that only for an anodal ratio of 9:1 do all the fibres propagate unidirectional action potentials. Increasing the current results in the successive blocking of larger fibres. During the in vitro experiments the range of blocking currents which most agreed with the model predictions was achieved with an anodal ratio of 9:1. For this anodal ratio, the model predicts the minimum current needed to block range from 600μA., for blocking the larger axons, to 1400μA, for blocking the smaller axons. The experimental results show blocking has been achieved with current thresholds within this range in 60% cases. In the other 40%, more current was needed than predicted. This was not surprising in experimental situation.

Minimum current to block axons with an anodal ratio of 9:1

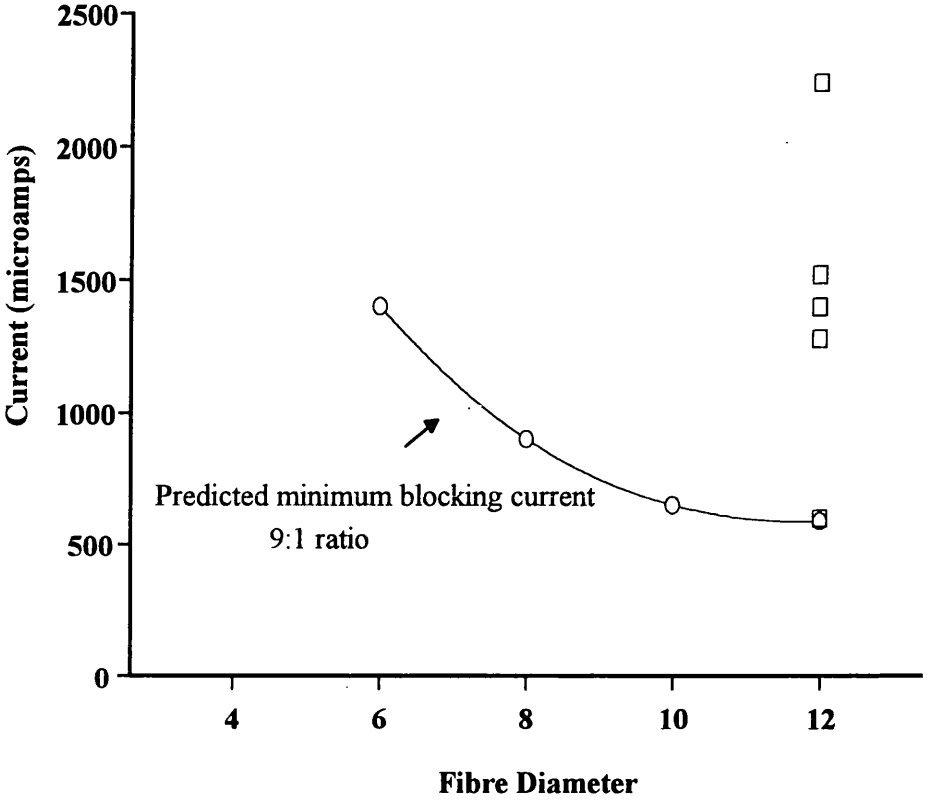


Figure 22 - The minimum current needed to produce blocking during the sciatic nerve experiments are compared to the predicted blocking currents for an anodal ratio of 9:1. Blocking was achieved experimentally with current thresholds at or above the predicted minimum.

Key:

0 - model predictions

□ - experimental results

Anodal current duration

The pulse duration largely determines which axons will avoid the blocking action of the anodes. Any action potential arriving at an anode will be blocked if a sufficiently stronger anodal current is flowing. The time of arrival of action potentials will be determined by the axonal conduction velocity and the distance from the point of excitation to the anode.

The model was calculated for pulse durations of 300μs and 500μs. The experimental results show that block was also possible with longer and shorter pulse widths. The range of pulse duration which were successful to achieve an anodal block during the 11 experiments in rabbit sciatic nerve can be seen at table 4. Anodal block was achieved with pulse widths that varied from 60μs to 1000μs. However, block was achieved more frequently at 300μs.

The surprising results is that block was achieved with 60 μs. It is expected that fibres as fast as 30 m/s should escape past the blocking anode with this duration, as can be seen in table 12, in the discussion. These escape volleys are not seen.

Subsequent experiments showed that the actual duration of current flow was longer than the nominal value quoted this far. Figure 23 - A shows current monitored during testing of the stimulator with a purely resistive load. The current is almost exactly rectangular and the actual duration is very close to the nominal duration. However, when the current was connected to a load comprising of the electrode plus nerve the form of the current changed significantly (figure 23 - B). The current pulse became rounded and an unexpected "tail" appeared at the end of the pulse. This slow decay of the currents lengthens the duration of the pulse by approximately 100 μs. So, the unexpected block with nominal duration's of 60 μs is probably explained by an actual duration of about 160 μs.

The slow current decay has a time constant of about 60 μs and this probably also explains why anode break excitation was never a problem with this stimulator. The stimulator had additional capacitors which could be switched in to increase the unexpected time constant. These were never needed.

In conclusion, the experimental results obtained during stimulation of the rabbit sciatic nerve generally agreed with the model predictions. Blocking was achieved more frequently at pulse duration's of 300μs and for anodal ratios of 9:1. However, more current was needed to achieve block than predicted, but these results were already expected because the model fails to consider several features such as the presence of other tissues like blood which may disturb the external electrical field of a nerve axon. In addition, the presence of other nerve axons nearby, which were not modelled, should decrease the available current for each fibre. A more detailed discussion about the factors that may interfere with nerve excitability will be presented later in this thesis.

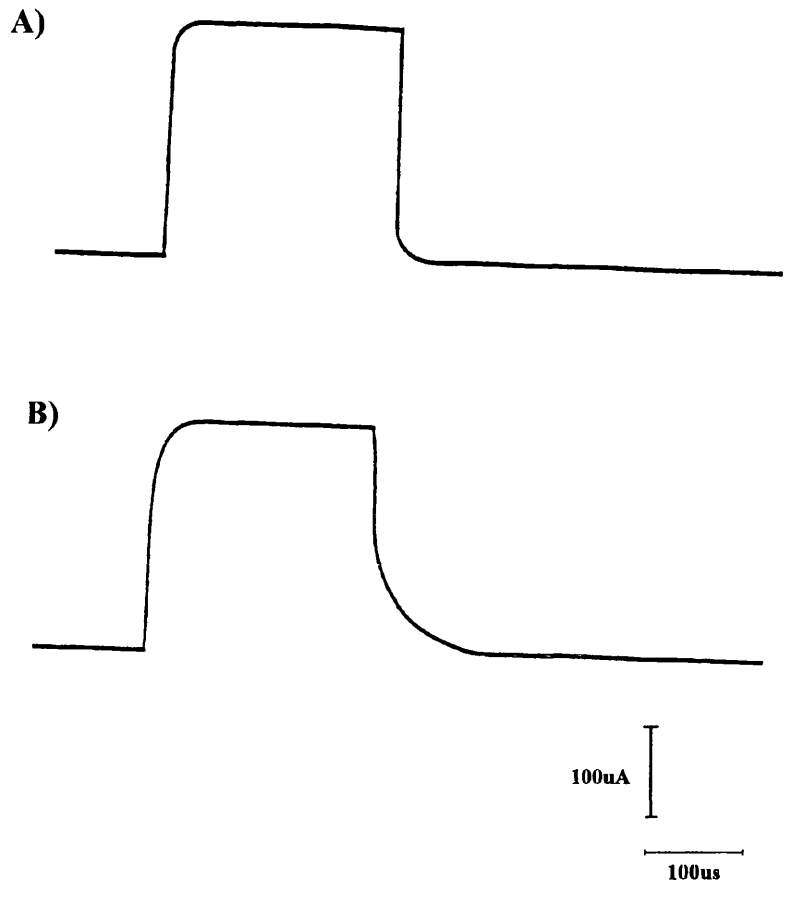


Figure 23 - Current output monitored during testing of the stimulator.

A - stimulator output with a purely resistive load. The current is almost exactly rectangular and the actual duration is very close to the nominal duration.

B - stimulator output connected to a load comprising of the electrode plus nerve. The current pulse became rounded and an unexpected "tail" appeared at the end of the pulse. This slow decay of the currents lengthens the duration of the pulse by approximately 100μs.

CHAPTER SIX
SELECTIVE ACTIVATION OF AUTONOMIC MOTOR FIBRES: IS IT POSSIBLE FROM THE ESCAPE ANODE?

Chapter 6 - Selective Activation of Autonomic Motor Fibres: is it possible from the escape anode?

The second prediction of the modelling studies is that it may be possible to regulate the anodal current at the escape end of the cuff electrode to provide for selective blocking of the larger diameter axons whilst allowing for escape of action potentials in the smaller axons.

The experiments presented in chapter 5 observed the effects produced at the blocking anode, but did not offer any substantial information about the behaviour of axons at the escape end. In those experiments it was observed that the tripolar cuff electrode is able to produce a total block of the action potentials at the blocking anode. However, sometimes a more specific action is required, such as when the tripolar cuff electrode is placed on a spinal root. If the blocking anode is located proximal to the root, it may block the propagation of action potentials in afferents towards higher levels of the spinal cord and CNS. However, there is no guarantee that a selective blocking would happen at the escape end. In the particular case of stimulation of the sacral roots that innervate the lower urinary tract, it would be interesting if this selective blocking could be achieved. During conventional bipolar sacral root stimulation, the somatic axons which innervate the external urethral sphincter are activated at lower current thresholds than the autonomic axons which innervate the bladder. The urethral sphincter would thus contract prior to bladder and this could lead to an unphysiological micturition process. In this case, responses of axons at the escape end of the cuff are very important. Accordingly, the experimental arrangement was changed to make it possible to test the probability of selective blocking of the larger somatic motor fibres and

the selective activation of small autonomic motor fibres during bipolar and tripolar stimulation.

The ventral sacral roots contain a mixed population of somatic and autonomic fibres. Consequently, the ventral sacral root is appropriate to study the effect of stimulation in larger and smaller motor axons. The effect of ventral sacral root stimulation in the lower urinary tract was observed in 46 adults New Zealand male rabbits. As in chapter 5, histological studies of the sacral root were made to confirm the appropriateness of their range of axons comparing to the model studies. Also, anatomical and histological observations of the lower urinary tract in rabbits were performed.

6.1 - Methods

6.1.1 - Histological methods

Sacral root

The mathematical model developed by Fitzpatrick predicted the responses of axons between 4 and 12 μm in diameter to tripolar stimulation. Histological studies of the sacral root of rabbit were performed to establish if their range of axon diameters was within the range studied in the model.

Light Microscopy

Sections of rabbit's sacral root were fixed in Carson's phosphate buffered formalin (Carson et al, 1973), dehydrated in graded alcohol and embedded in Paraplast. Ten micrometers serial sections were cut using a Leica 2050 microtome. The sections were then stained with phosphotungstic acid haematoxylin (P.T.A.H.) or Miller's elastic stain

(Miller, 1971) and Van Geison (1889). The stained sections were examined using a Zeiss Axiophot microscope and photomicrographs taken on Kodak Ektachrome 64 (tungsten). The total nerve fibre was reconstructed in a photomontage from where the total diameter of each axon was measured and counted as described in chapter 5. The results are presented in a frequency distribution histogram to be compared to the range of axons studied in Fitzpatrick model.

Urethral Sections

In some of the experiments it was very difficult to record the urethral external sphincter activity in rabbits. Thus, anatomical and histological studies were carried out to confirm the sphincter position.

Urethral sections were stained with PTAH (phosphotungstic acid - haematoxylin), according with the technique described by Shum and Hon (1969) and Bancroff and Cook (1984). According to these authors the proportion of phosphotungstic acid to haematein is important for good results (0.9%/0.08%). They also state that premordanting is unnecessary and that long staining at room-temperature gives slightly more precise results than shorter staining at 56°C. The stain may be used repeatedly and should be filtered after use.

Three solutions were used: 0.25% potassium permanganate, 5% oxalic acid and the stain. The stain is prepared as follow: dissolve 0.08g haematein in 1ml distilled water, and grind to a "chocolate paste". Then, dissolve 0.9g phosphotungstic acid in 99 ml distilled water and mix with the haematein solution. Bring to boil, cool and filter.

After preparing the solutions, the sections were take from water and the mercury was removed if necessary. Then, the section was left for 5 minutes in 0.25% potassium permanganate, washed in running

water and bleached with 5% oxalic acid until the section is clear. Once more, the section was washed well in running tap water, then stained with PTAH during 12 to 24 hours at room temperature and dehydrated rapidly, cleared and mounted in D.P.X.

Dehydration should be rapid since water and alcohol may remove some stain. A degree of differentiation can be achieved during dehydration. The final colour balance in the slide may vary.

Generally, skeletal and smooth muscle stain blue with PTAH as does red blood corpuscles, nuclei, fibrin, elastin, myelin amongst others. Collagen stains brick red with PTAH.

6.1.2 - General Experimental Design

This section gives a general overview of the experimental design.

Experiments were carried out in 46 deeply anaesthetised adults male New Zealand white rabbits. Their weights varied between 1.9 Kg and 5.25 Kg, averaging 3.2 Kg. All the procedures were in accordance with granted Home Office licences (personal licence 60/04610 - project license 60/01193). However, 8 experiments were not completed (17%): 4 rabbits (8.7%) died due to anaesthetic problems, there were problems with the laminectomy in 3 rabbits (6.5%) and the electrode did not work in 1 case (2.1%). In these last 4 experiments neither bladder nor sphincter contraction were achieved.

After a low lumbar-sacral laminectomy with dorsal rhizotomy a unilateral sacral root (S_2 or S_3) was stimulated at a frequency of 20 Hz. The stimulator set-up described in chapter 5 was also used here.

Experiments were performed to:

1 - Test the excitability of larger somatic motor fibres. Skeletal muscle activity was recorded using averaged EMG from those muscles innervated by the same stimulated root.

2 - Test the possibility of selective activation of the small autonomic motor fibres. In addition to EMG, bladder and urethral pressure were recorded during bipolar and tripolar ventral sacral root stimulation. Bladder and urethral activity were evaluated by conventional urodynamic methods.

In the previous experimental series, nerve excitation was recorded directly as compound action potentials in the nerve trunk. This approach was changed because of the difficulties associated with dissection of the small muscle nerves in the hip and tail region. Similar problems were expected with the nerve supply to the bladder and external urethral sphincter. Indirect methods were used to identify nerve activity, i.e. intramuscular EMG recording to identify activation of skeletomotor axons supplying the hip and tail, intravesical pressure to identify activation of autonomic axons supplying the bladder and intraurethral pressure to observe activation of axons supplying the external sphincter.

The experiments were divided as follows;

- 5 initial experiments to set-up anaesthetic doses and to study bladder and urethral sphincter behaviour in rabbits during several levels of anaesthesia. However, throughout the project, additional observations were made on the changes in rabbit's lower urinary tract behaviour related to anaesthesia.
- 5 experiments to observe the acute effects of rhizotomy on bladder and sphincter behaviour. Urodynamic evaluations were performed before and after rhizotomy to observe any acute change that could interfere with the stimulation response.

. 28 experiments to observe the effects of bipolar and tripolar stimulation: 15 compared the response of the smooth muscle in the bladder to the first skeletal muscle to be stimulated by the same sacral root and 13 compared the bladder response to the urethral external sphincter response.

Rabbits were sacrificed at the end of the experiments by an anaesthetic overdose of Halothane.

6.1.3 - Anaesthesia

The rabbits were anaesthetised with a combination of Diazepam (Valium^R 10, Roche) in a dose of 1.5 mg per Kg injected intraperitoneally, and fentanyl citrate 0.315 mg per ml + fluanisone 10 mg per ml (Hypnorm^R, Jansen Animal Health) 0.15 ml per Kg injected intramuscularly.

A tracheostomy was performed and when necessary anaesthesia was supplemented with a mixture of Halothane (Fluothane^R, Imperial Chemical Industries plc) in 20% of O_2 and 80% NO_2 via a tracheal tube. The halothane was used at concentrations up to 4%. The level of anaesthesia was assessed by testing the corneal reflex and flexor withdrawal reflexes in response to paw pinch. No surgical procedures were performed before the animal was deeply anaesthetised. Anaesthesia was maintained by means of the gaseous mixture described above because it was found easier to control. The rabbits' conditions were continuously monitored and normal physiological values of temperature, respiration rate, blood oxygenation and CO_2 pressure were maintained.

6.1.4 - Surgical Aspects

Tracheostomy:

With the animal in prone position and under deep anaesthesia, a classical tracheostomy was performed. The fur on the neck was removed from the jaw to the sternum with clippers. The neck was hyper-extended. A anteromedial incision in the skin from the suprasternal notch to the hyoid bone allowed exposure of the tracheal region. The skin flaps were retracted and the trachea was palpated under the anterior muscles of the neck. Dissection of these muscles in the middle line exposed the trachea. The lateral regions of the trachea were cleared by blunt dissection, and two linen threads were passed round it. The threads were separated, one being positioned proximal and the other distal to the tracheal region to be incised. After careful cauterisation to prevent bleeding, the trachea was partially incised between two cartilage rings. The distal ring was gripped in curved Halstead mosquito forceps and a suitable glass tracheal cannula was inserted. The tracheal cannula was carefully fixed in place using the linen threads. After checking if the airways were clear, and the animal was been satisfactorily ventilated, the skin incision was closed with Mitchel clips and the neck extension released.

Laminectomy + Rhizotomy:

A low lumbar-sacral laminectomy (L_7-S_4) was performed to expose the sacral roots, with the animal in supine position. The fur on the rabbit's back was shaved from the first lumbar vertebra to the base of the tail. A medial incision in the lumbo-sacral region (from L_4 to S_5) exposes the superficial lumbodorsal fascia, which was opened to expose the tips of the spinous processes of the lumbo-sacral vertebrae (figure

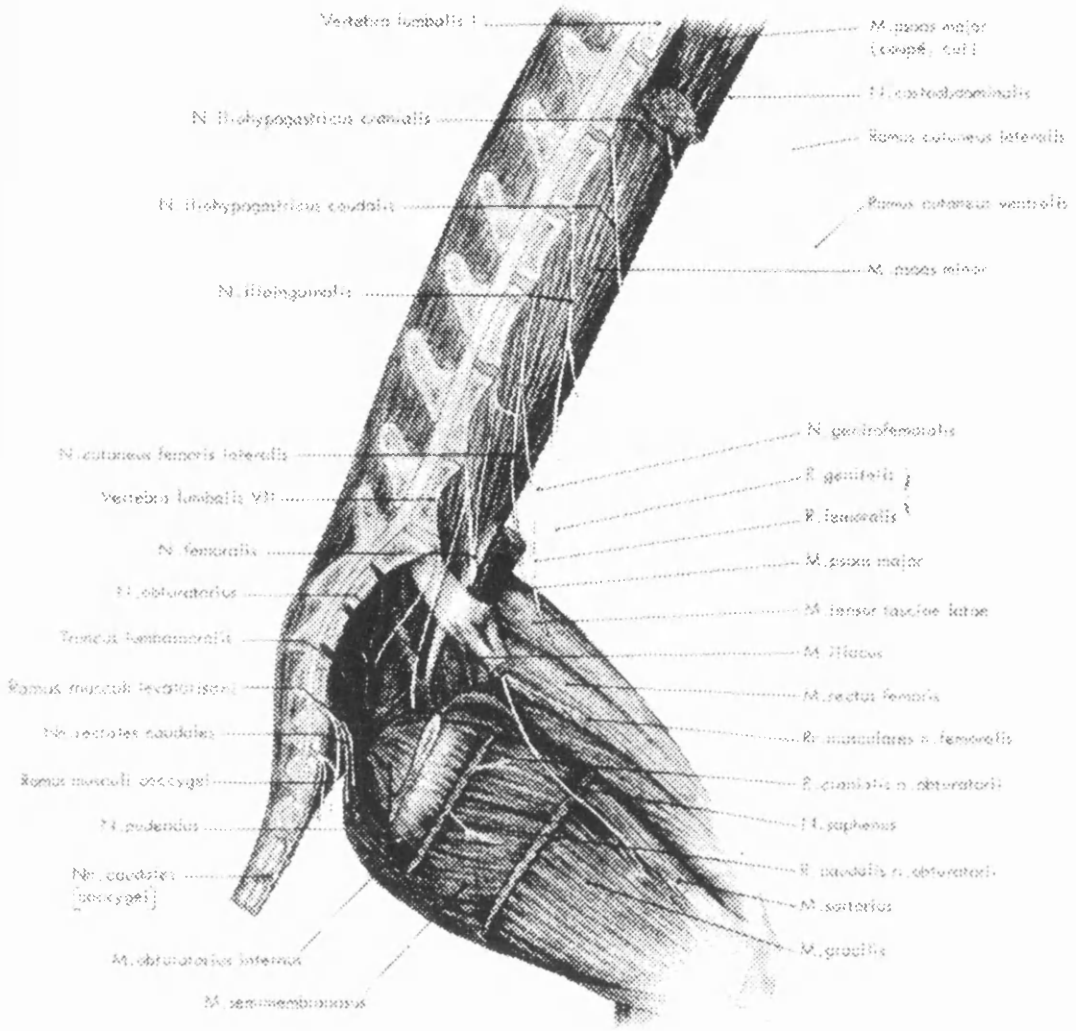
25). Categorical cauterisation of all bleeding points was performed at this stage.

Under microscopic dissection, the roof of the vertebral canal was removed from L_7 to S_4, thus exposing the spinal cord (figure 26). The dura mater is opened and reflected to expose the lumbo-sacral roots (figure 27). Dorsal sacral rhizotomy and cordectomy exposed the ventral roots S_2 to S_4 .

Bleeding during laminectomy was difficult to control and was a significant problem in some experiments. Compressing the bleeding point after applying absorbable gelatine sponge (Sterispon^R No.3, Allen and Hanburys Ltd) stopped the bleeding on most occasions. A frame which held and lifted the animal also helped to minimise bleeding by reducing the abdominal pressure in the supine position (figure 24). After bleeding was controlled, the sacral roots were identified.

Dorsal roots were cut to facilitate exposure of the ventral roots and placement of the electrode. The rhizotomy combined with cord section at low-lumbar level also eliminated any reflex effects due to accidental stimulation of afferents. Brief periods of bipolar stimulation were useful in identifying roots as dorsal or ventral. Stimulation of dorsal roots did not produce muscle contractions, whilst stimulation of ventral roots did.

Identification of the precise spinal level of ventral roots was not always possible in the rabbit. Each root was stimulated in turn and the experiment began with stimulation of the root which produced the strongest bladder and urethral sphincter contractions. This was generally identified as S_2.

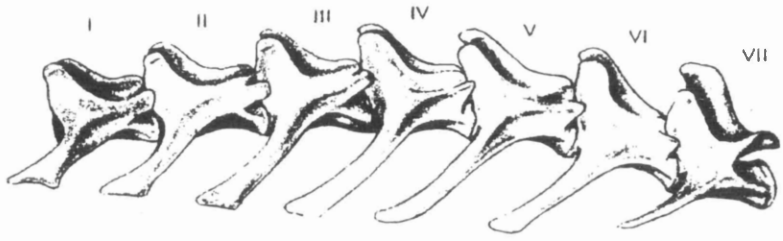


PLEXUS LUMBOSACRALIS.
Plexus lumbo-sacré.
Lumbosacral plexus.

VERTEBRAE LUMBALES.

Vertèbres lombaires.

Lumbar vertebrae.

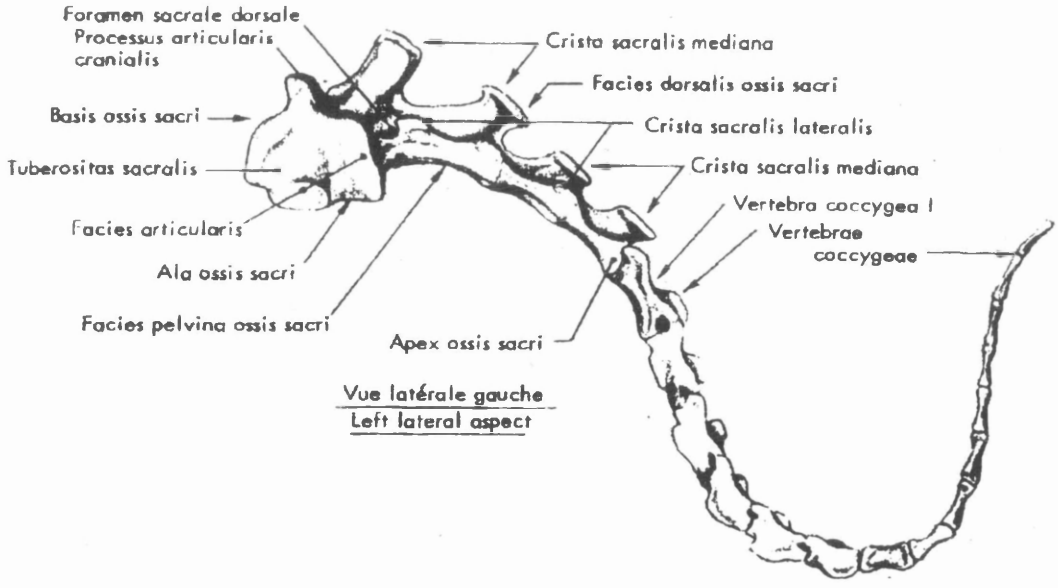


Vue latérale gauche
Left lateral aspect

OS SACRUM ET VERTEBRAE COCCYGEAE.

Sacrum et vertèbres coccygiennes.

Sacrum and coccygeal vertebrae.



Vue latérale gauche
Left lateral aspect

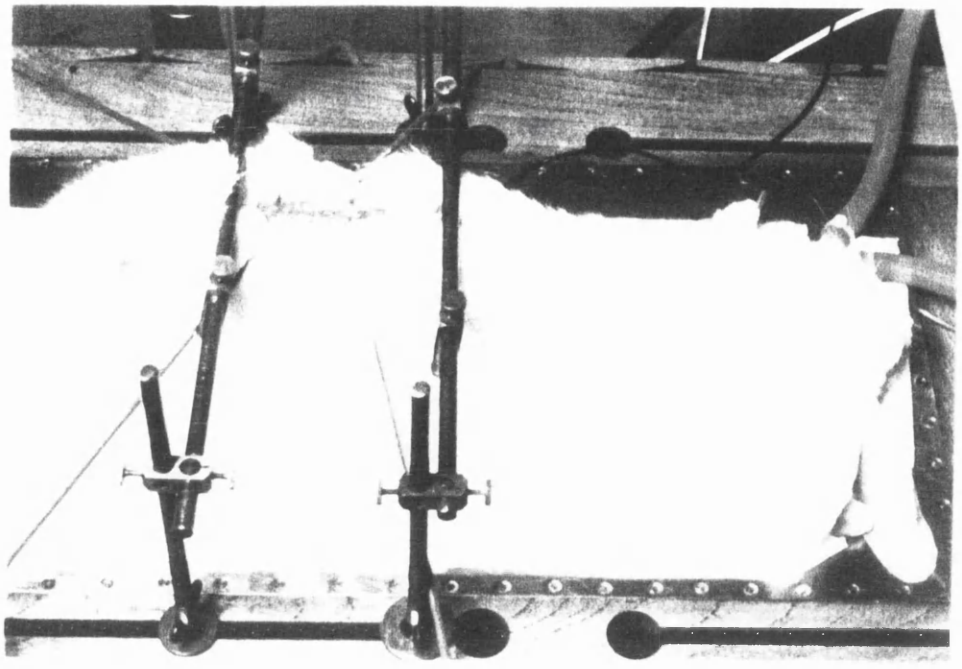


Figure 24 - Rabbit in the experimental frame, which held and lifted the animal and helped to minimise bleeding during laminectomy by reducing the abdominal pressure.

Figure 25 - Spinous processes of the lumbo-sacral vertebrae.

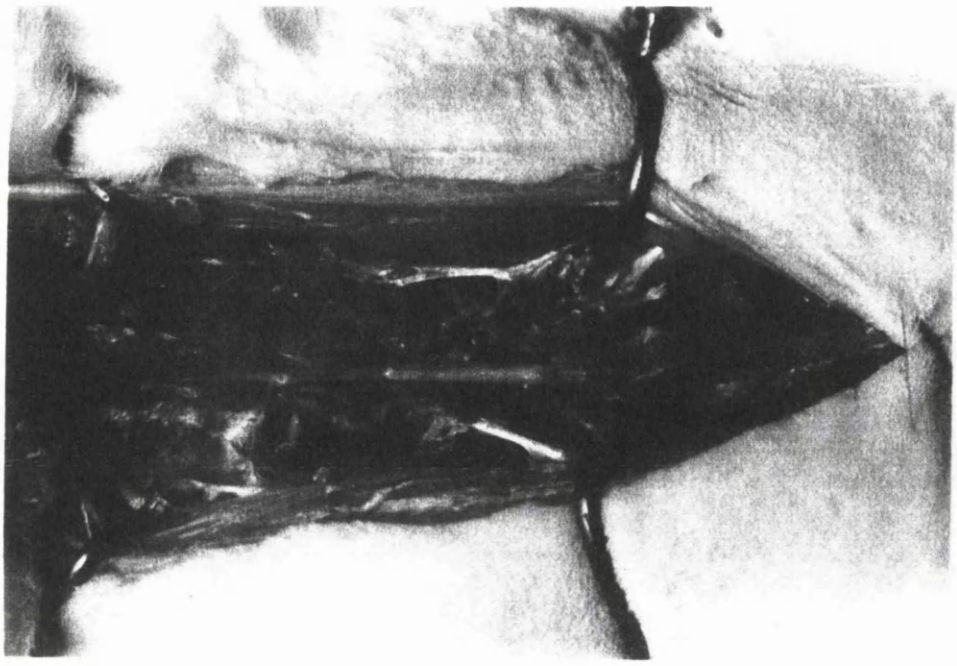


Figure 26 - The roof of the vertebral canal was removed from L_7 to S_4, exposing the spinal cord.

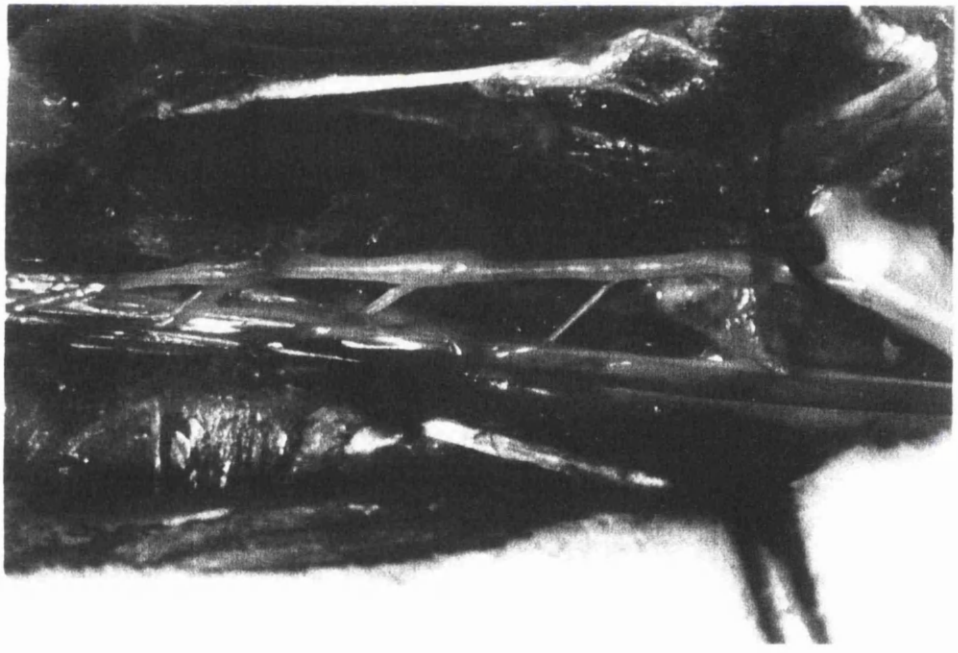


Figure 27 - The dura mater is opened and reflected to expose the lumbo-sacral roots. In the figure, the spinal cord was retracted to allow a better view of the roots.

6.1.5 - Stimulation Methods

The same stimulator set up as described in chapter 5 was again used here. After root identification, the tripolar electrode was placed at S_2 or S_3 root, unilaterally, and the field was covered with paraffin oil warmed to 37°C.

A single sacral root (S_2 or S_3) was stimulated at a frequency of 20 Hz. The pulse width was kept constant at 300μs. The current intensity was increased progressively until contractions were observed in the muscle of the tail. Subsequent increase in current intensity continued until the urethral sphincter and the bladder were also activated. The stimulation was delivered in bursts lasting 2- 4 seconds repeated every 20 seconds, until threshold was achieved for tail muscle, bladder and urethral sphincter in each experiment.

Initially, the stimulation was bipolar. The current thresholds for the skeletal muscle (tail or leg), external urethral sphincter and bladder were recorded. Then, the stimulation was switched to tripolar mode and the threshold measurements were repeated. After the minimum current to stimulate the tail muscle, the bladder and the urethral sphincter was determined in bipolar and tripolar mode, the stimulation was switched off, and following a rest period of approximately 15 minutes, the experiment was repeated to test reproducibility, i.e., the stimulation started at the minimum currents to stimulate as identified above and the threshold values were confirmed.

6.1.6 - Urodynamic Methods and Electromyography

Cystometry

Cystometry is the measurement of bladder pressure as a function of volume during filling and emptying. The cystometrogram (CMG) is the graphic representation of cystometry. It is one of the most commonly used ways of monitoring bladder activity.

To perform cystometry, the rabbit urethra was catheterised with a 7Fr 30 cm triple lumen urodynamic catheter (Cook Urological^{R}). This is a paediatric catheter but its size and configuration were adequate for use in rabbits. This catheter has 3 side openings. The distances between the openings are calculated so that when the catheter is inserted, two openings are positioned in the bladder while the third opening is located in the urethra. Thus, it is possible to fill and record bladder activity by two openings and record urethral activity by the third and most distal opening. The catheter has raised ridges near the opening. These can be palpated through the skin to allow more accurate positioning of the catheter. Alternatively, a triple lumen urodynamic balloon catheter was used (Cook Urological^{R}). The advantage of the balloon catheter is that it allows the position of the catheter to be fixed in the bladder. However, before sphincter pressure is measured the balloon must be deflated and the catheter repositioned. Figure 28 shows details of these urodynamic catheters. The appropriate catheter's position is shown in figure 29.

The bladder was slowly filled with warmed physiological saline, at a constant volume per minute ratio, which is controlled by an infusion pump. The intravesical pressure was recorded by connecting the catheter to a Elcomatic EM 751 pressure transducer which was connected to a Neurolog NL 108 pressure amplifier.

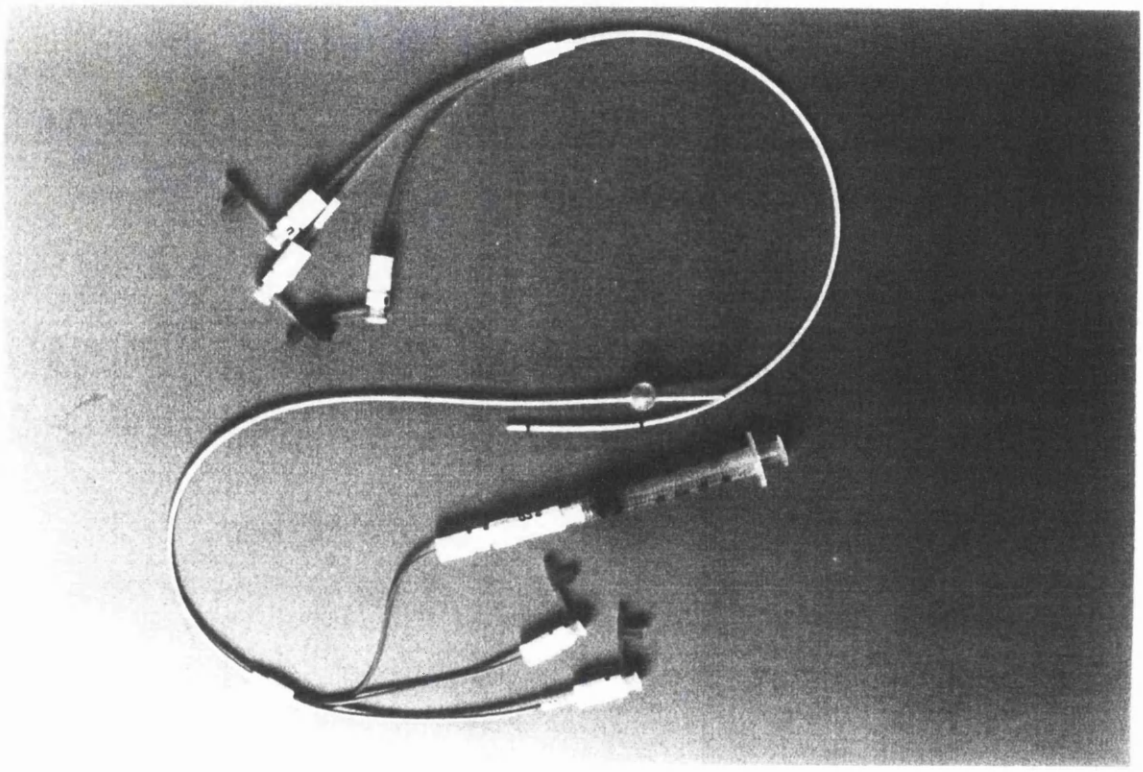


Figure 28 - Triple lumen urodynamic catheter and triple lumen balloon urodynamic catheter. These catheters are specially designed with 3 lateral openings to permit filling of the bladder while recording bladder and urethral activity. As can be seen from the figure, the position of the openings comes already marked according to their connections at the end of the catheter. This allows to position them at the bladder (B) and urethral (U) level, and fill the bladder (fill).

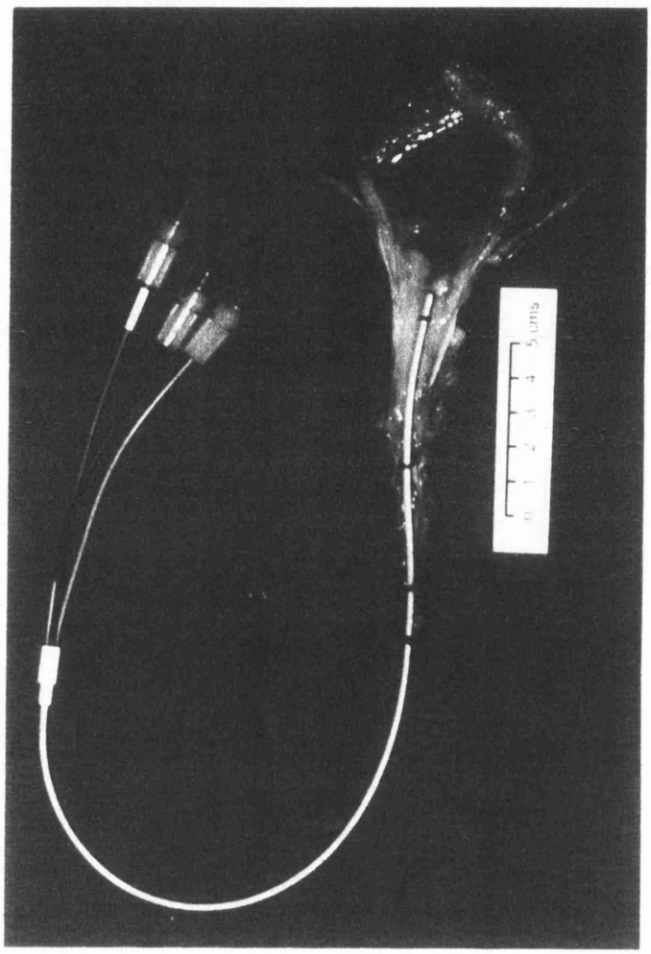


Figure 29 - Triple lumen urodynamic catheter ideally positioned in an open lower urinary tract of a rabbit.

The CMG was recorded after initial anaesthesia at the beginning of the experiment. It was repeated after the laminectomy and the rhizotomy and during both bipolar and tripolar stimulations.

Urethral pressure

The urethral pressure may be monitored as the pressure profile along the urethra or by the measurement of the pressure at a single point. During the experiments the urethral pressure profile was used to find the urethral external sphincter, the urethral pressure was then measured at this location during stimulation.

The urethral pressure profile is the measurement of pressure at different points along the urethra. During the experiments, the triple lumen urodynamic catheter was positioned in the bladder. The bladder was filled with physiological saline and the catheter was slowly and smoothly withdrawn while the pressure was recorded. The point where maximum pressure occurred corresponds to the position of the external urethral sphincter. Figure 30 illustrates the pressure profile in the bladder and urethra.

During the experiments, it was not always easy to measure the urethral sphincter activity in the rabbit. Therefore, the positioning of the catheter in the urethral external sphincter was also tested in other ways. The catheter has a ridge on it near the opening for sphincter pressure measurement. In the rabbit, this ridge could be palpated through the skin and it was used to guide the catheter into position by correlation with the anatomical sphincter position. Another technique was to look for a rise in the sphincter pressure during urodynamic evaluation. This was achieved by exerting external pressure at the point where the sphincter

Diagram of an urethral pressure profile in the male

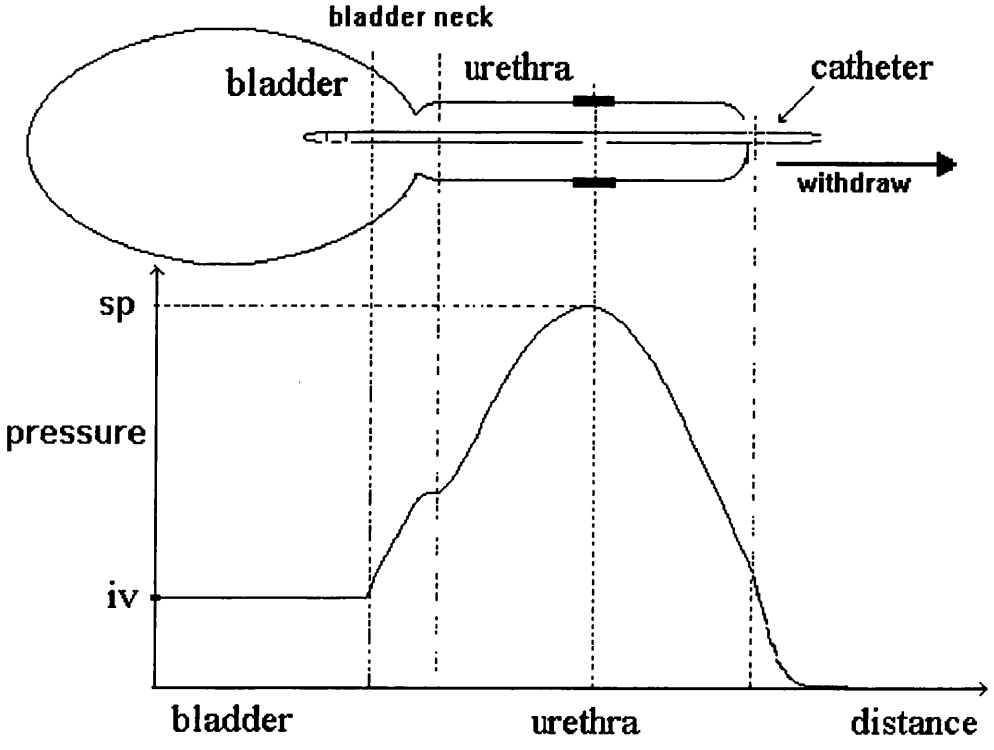


Figure 30 - Diagram to show the relationship of the pressure profile in the bladder and urethra. The pressure inside the bladder and the urethra is measured by means of the lateral opening in the 3 way urodynamic catheter which is connected to the pressure transducers (see methods). This catheter is positioned initially in the bladder, then withdrawn through the urethra, measuring the pressure on different points of the circuit. The broken lines show the point which the pressures are being measured. The bladder pressure usually starts at around 10cm H_2O, this pressure corresponds to the abdominal pressure. In males, it is expected a rise in pressure at the level of bladder neck, as show above. The point of maximum pressure corresponds to the position of the external urethral sphincter.

iv = intra-vesical pressure

sp = sphincter pressure

was expected to be. The anatomical observations and histological studies also helped to define the sphincter position.

Electromyography (EMG):

Activity in skeletal muscles innervated by S_1- S_3 sacral roots was recorded by stimulating the root and observing which muscles contract. Generally, muscles near the base of the tail (coccygeal muscle) were activated. Bipolar wire electrodes were inserted near the base of the tail via a hypodermic needle, which was subsequently withdrawn. The EMG potentials recorded between the wire electrodes were differentially amplified x1000, and filtered when necessary 10 - 500 Hz. The first signs of muscle contraction were identified as small muscle action potentials time locked to the stimulus seen in post stimulus average.

6.2 - Results

6.2.1 - Sacral root

Figure 31 shows a photomontage of a section of a sacral root (S_2) magnified 786 times. The larger thickly myelinated axons can still be seen easily. The smaller more thinly myelinated axons were also seen as were many unmyelinated axons. Sometimes, larger and smaller axons seem to be distribute in groups seen elsewhere. A more detailed picture is presented in figure 32. At this particular point, the smaller, thicker and thinly myelinated axons are more numerous than the larger axons.

The axons were also counted and their diameters calculated as described to the tibial nerve, in chapter 5. The results are presented in a fibre-size histogram in figure 33. There is a larger number of axons between 4μm and 12μm in diameter. This confirms that the range of axons used in the model studies are well represented in the nerves to be tested in the animal model.

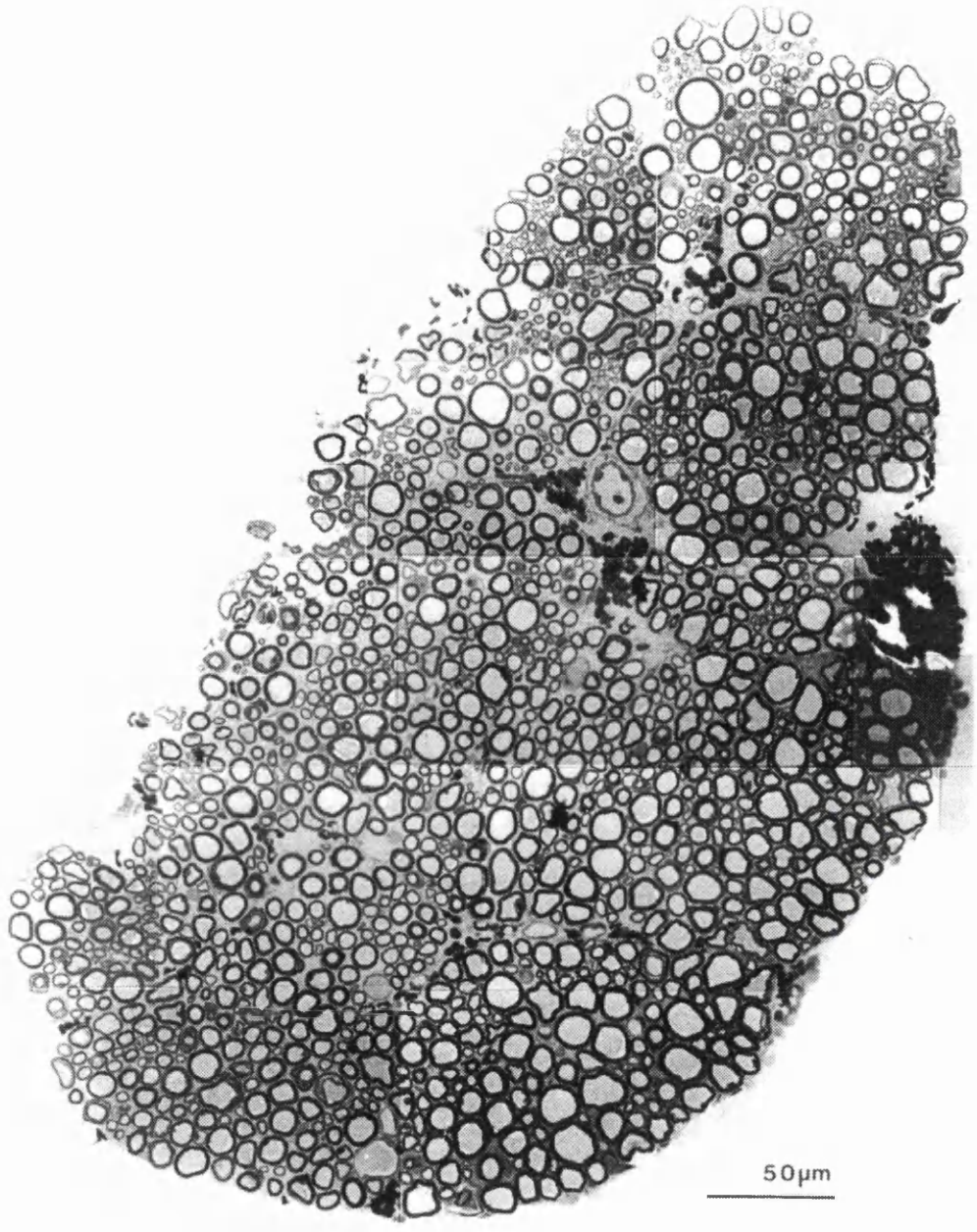


Figure 31 - Photomontage of the sacral root magnified x 786. The larger myelinated axons are easily found. Note also the larger number of small myelinated and unmyelinated axons.

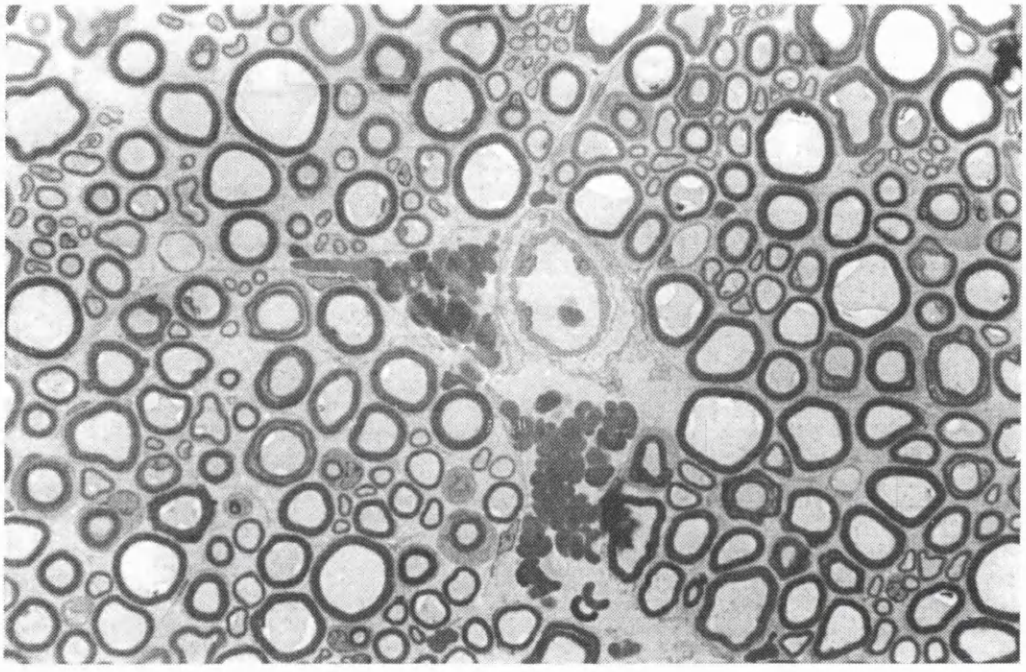
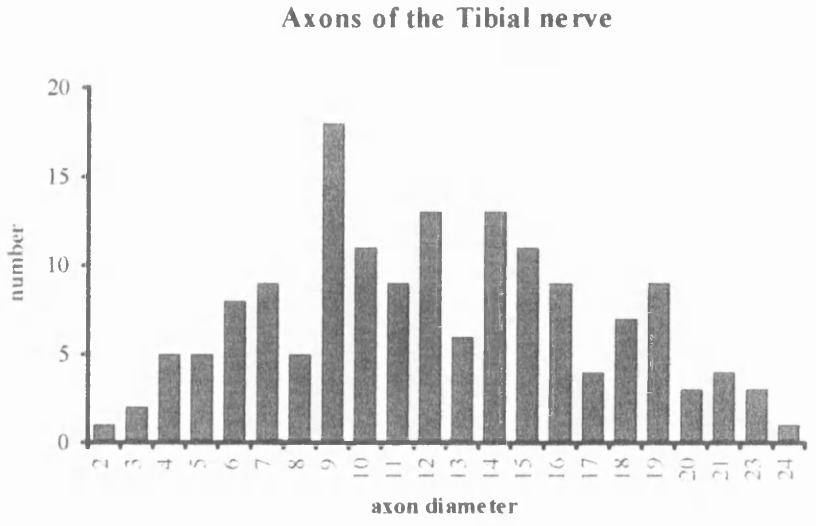


Figure 32 - Detail of a section of the sacral root shown in figure 31. Groups of larger myelinated axons and groups of smaller myelinated axons can be found. Some unmyelinated axons are also present.

Frequency distribution of axons in the tibial nerve and in the sacral root

A



B

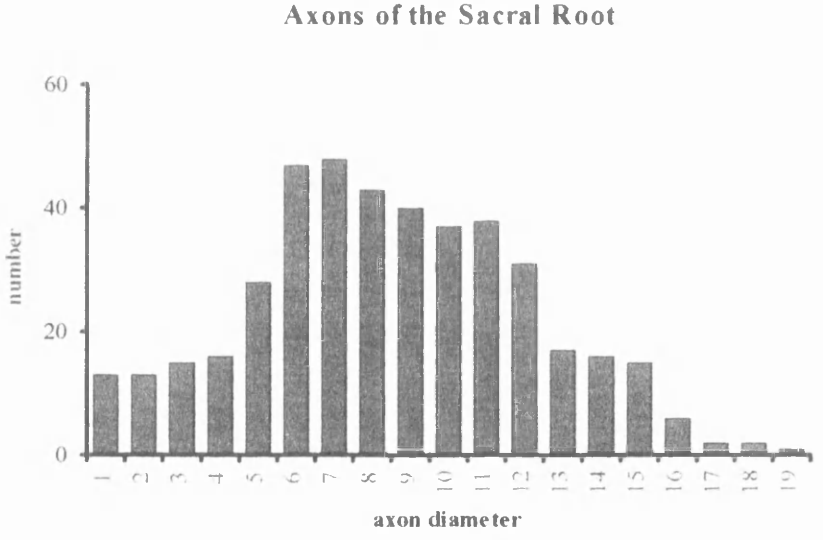
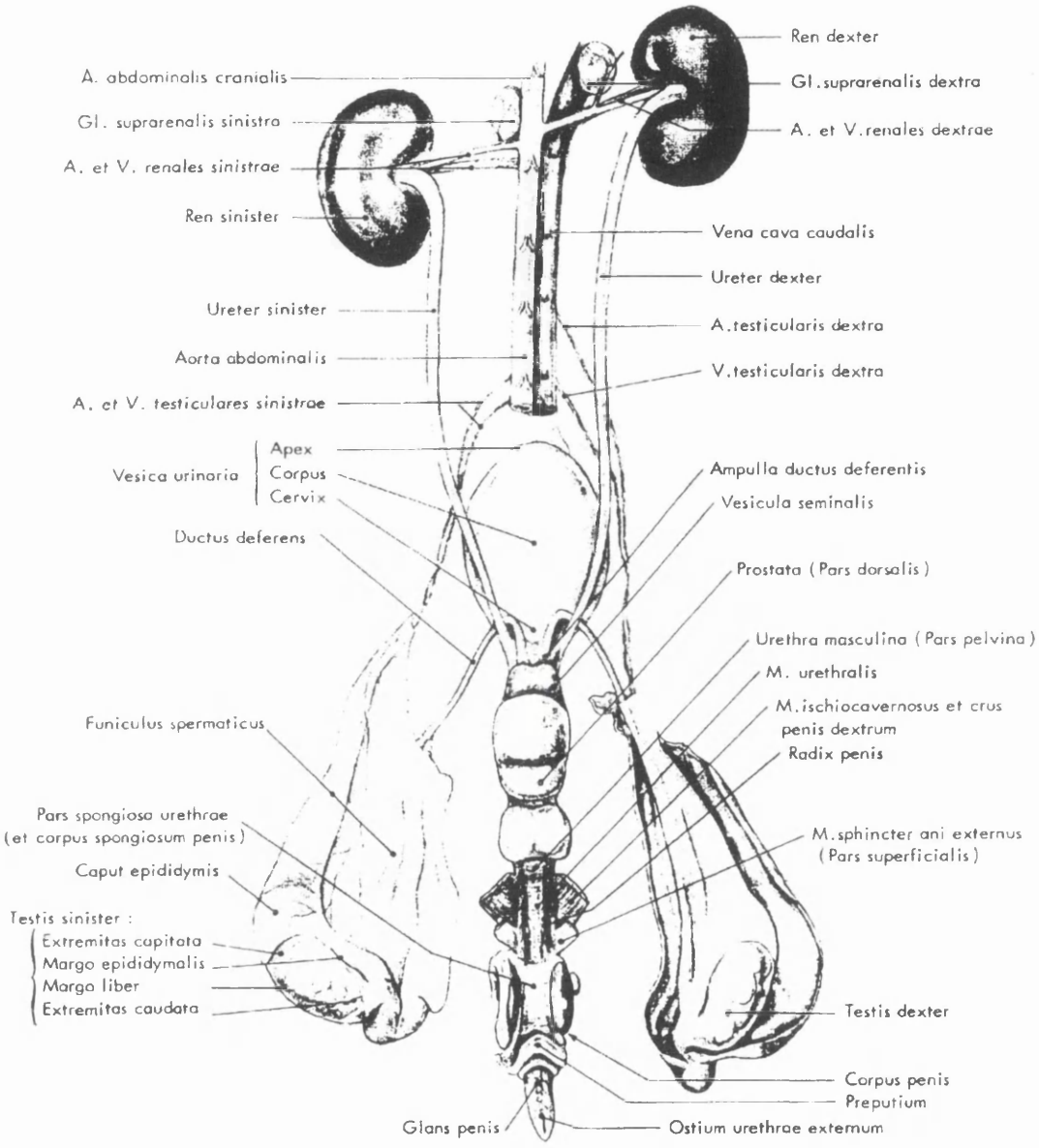


Figure 33 - Fibre-size histogram showing:
A- distribution of axons diameters in a section of the tibial nerve.
B - distribution of axons diameters in a section of the sacral roots.
The frequency distribution above confirms that the range of axons studied in the model, between 4μm and 12μm, are well represented in the nerves used during the test of the electrode.

6.2.2 - Urethra and External Urethral Sphincter

The urinary tract of a male rabbit is shown in figure 34. There is no evidence of large macroscopic differences between rabbit's urinary tract and other mammals. Nevertheless, the rabbit bladder is more elongated than the human bladder. Histological sections of the rabbit urethra at the level of the external sphincter and elsewhere along its length were also made. Urethral sections were stained with PTAH, as described in section 6.1.1 in Methods. As in humans, the rabbit urethra is mainly composed of smooth muscle fibres. At the level of the external sphincter the urethral sphincter is composed of striated muscle. Figure 35-A shows a low power micrograph of the rabbit urethra. As the section was taken at the level of the external urethral sphincter, the skeletal muscle is predominant. The smooth muscle layer is not in the plane of section, although some fibres can be seen in the lamina propria, beside the connective tissue and colloid material. Figure 35-B shows a higher power micrograph of the same section. The characteristic striations of the skeletal muscle cells of the external urethral sphincter can be seen beside the lamina propria.



ORGANA UROPOETICA ET GENITALIA MASCULINA (FACIES DORSALIS).

Organes urinaires et génitaux du mâle (vue dorsale).

Male urogenital organs (dorsal aspect).

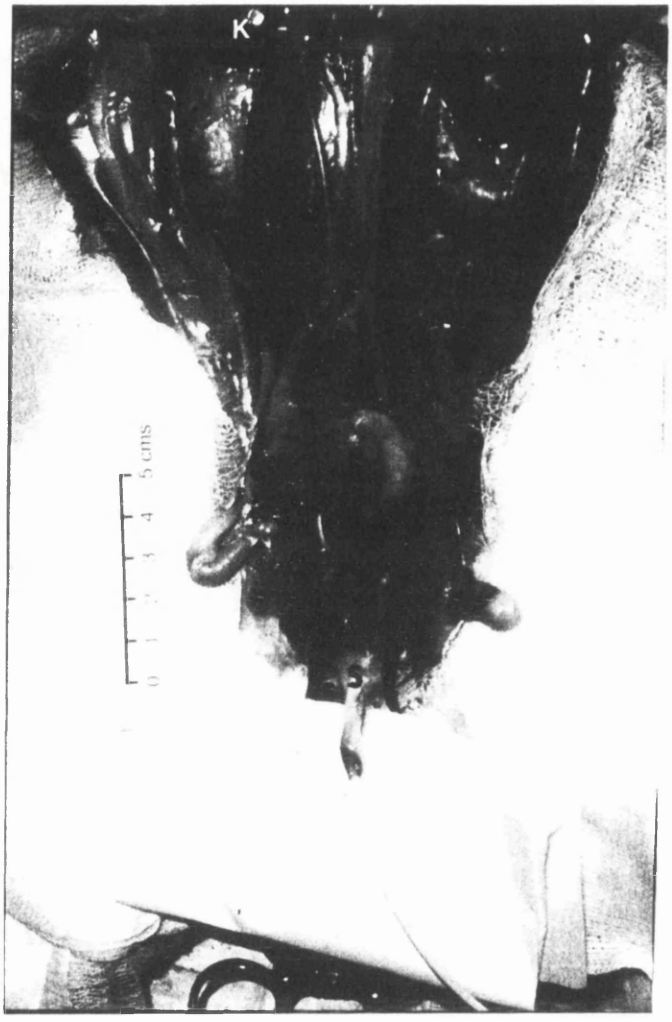


Figure 34 - Urinary tract dissected from a male rabbit.

K - kidneys

U- ureters

B - bladder

S - region of urethral sphincters

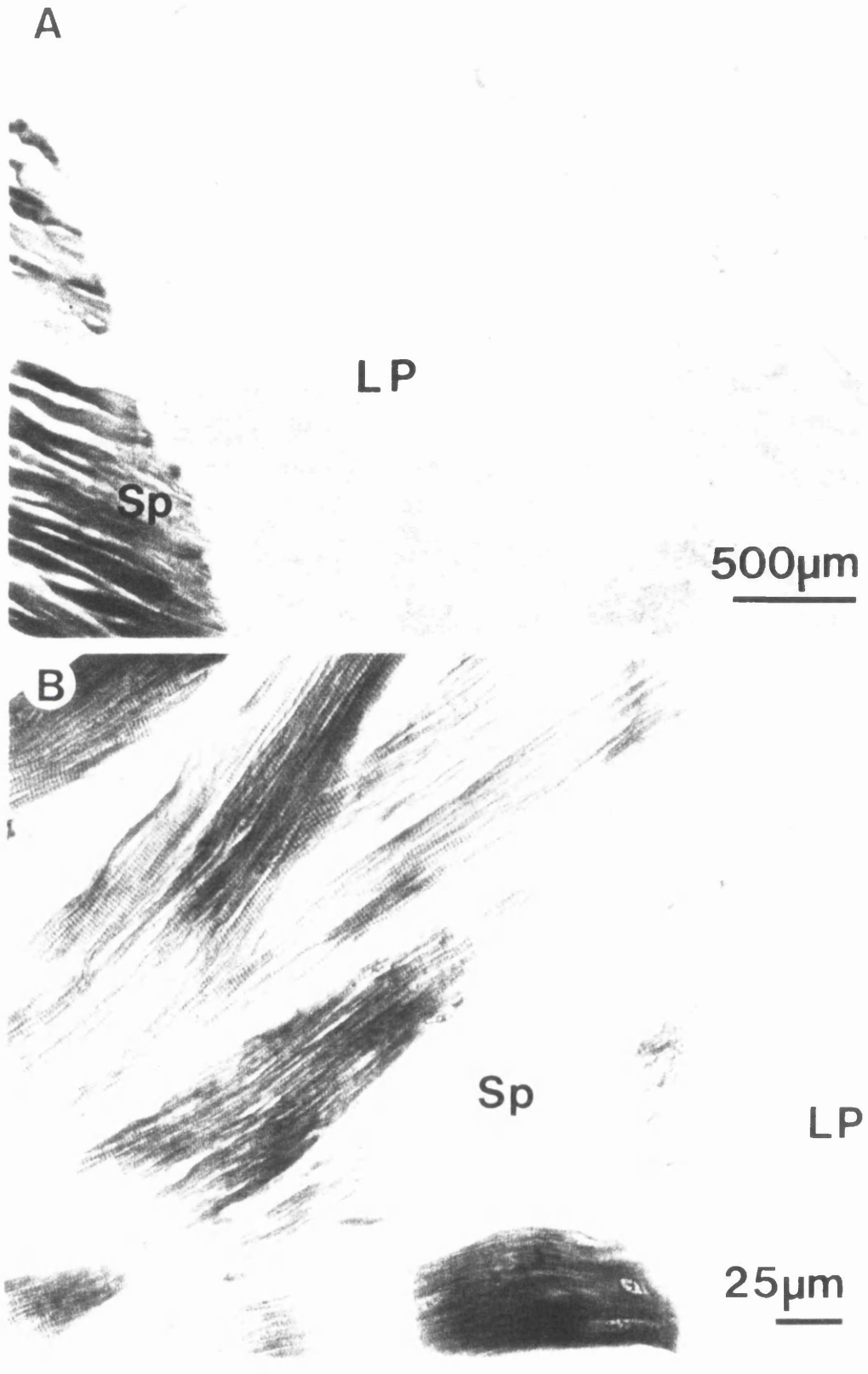


Figure 35

A - Low power micrograph of the rabbit urethra illustrating the external urethral sphincter (Sp) beside the lamina propria (LP). Stain PTAH.

B - In this higher power micrograph of the external urethral sphincter the striated pattern of the muscle cells can be seen.

6.2.3 - Lower Urinary Tract Behaviour during Anaesthesia

In all the experiments, rabbits were anaesthetised with a combination of diazepam, fentanyl and fluanisone, supplemented when necessary with a mixture of Halothane in 20% O_2 and 80% NO_2. Anaesthetic methods are described in section 6.13.

The lower urinary tract response during anaesthesia was tested in 15 experiments. Bladder and urethral behaviour, recorded by conventional urodynamic methods, were examined at different depths of anaesthesia. The parameters used to evaluate these responses were maximum bladder capacity, pressure to trigger micturition and maximum urethral pressure. The measurements were made thirty minutes after the injections of diazepam, fentanyl and fluanisone, and then thirty minutes after start halothane. These measurements were taken at halothane concentrations of 0.5%, 1%, 2% and 3%, at intervals of 30 minutes. With diazepam, fentanyl and fluanisone, reflex contractions from the detrusor and the urethral external sphincter were observed as was a light urethral resistance during catheterization. Almost no changes were observed until 1% Halothane was delivered, when the spontaneous contractions started to decrease. The urethral pressure profile seems to be abolished when the anaesthetic dose was greater than 2% of halothane. At halothane concentrations of 3% it was difficult to retain urine in the bladder. A dribbling of urine was almost always observed, then, and no spontaneous contractions were observed at the detrusor or at the urethra. Bladder capacity also becomes larger when the depth of anaesthesia is increased by 1% halothane. The maximum bladder capacity continues to increase with the depth of anaesthesia.

Figure 36 compares bladder response thirty minutes after administration of diazepam, fentanyl and fluanisone to bladder response at halothane concentrations of 2% to be compared. The bladder is still responsive after the injections of diazepam, fentanyl and fluanisone. Bladder pressure rises slightly at the beginning of the filling phase, reaching a maximum of 30 cm H_2O, at which point micturition occurs. Further filling results in another rise in the bladder pressure, and complete bladder evacuation, as described by Levin et al (1994). Maximum bladder capacity was 30 ml in this experiment. With a deeper anaesthetic level (Halothane 2%), bladder capacity increased to 60ml with decrease in bladder activity.

Figure 36 - Standard bladder and urethra response measured by means of urodynamic evaluation (cystometrogram and urethral pressure profile) at two different anaesthetic levels in rabbits.

A- Thirty minutes after the injections of diazepam, fentanyl and fluanisone. Experiments start with an empty bladder after any residual volume was removed. The bladder was slowly filled with warmed physiological saline, as described in section 6.1.6. Bladder and urethra pressures were monitored as a function of the volume. Bladder pressure rises slightly at the beginning of the filling phase, up to a maximum pressure of 30 cm H_2O. At this point, micturition starts. Further filling results in another rise and complete bladder evacuation. Maximum bladder capacity is 30 ml.

B - Halothane concentrations of 2%. With a deeper anaesthetic level, bladder capacity increased to 60 ml with a notable decrease in bladder and urethra activity.

Bladder and sphincter response according to the anaesthetic levels in rabbits.

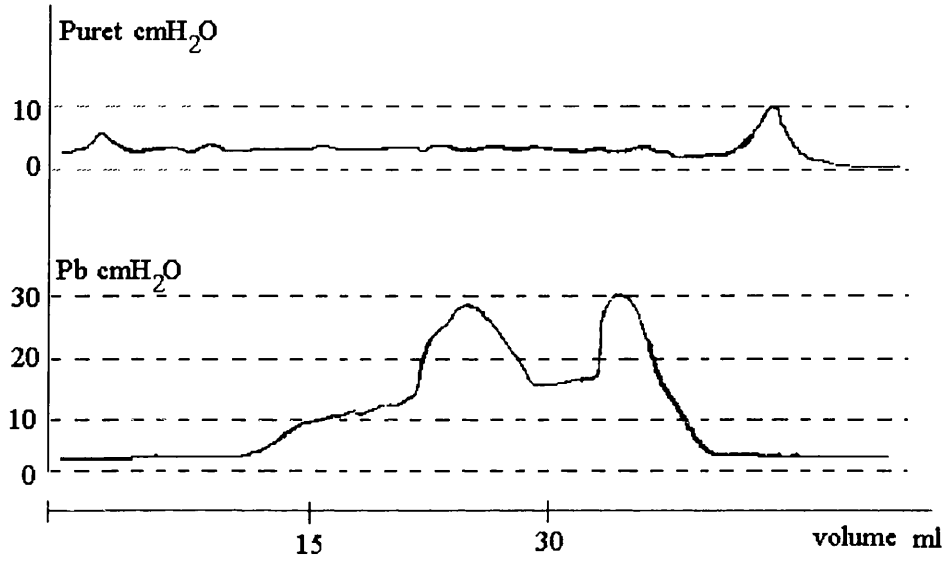


Figure 36 A- Thirty minutes after the injections of diazepam, fentanyl and fluanisone. Puret = urethral pressure, Pb = bladder pressure.

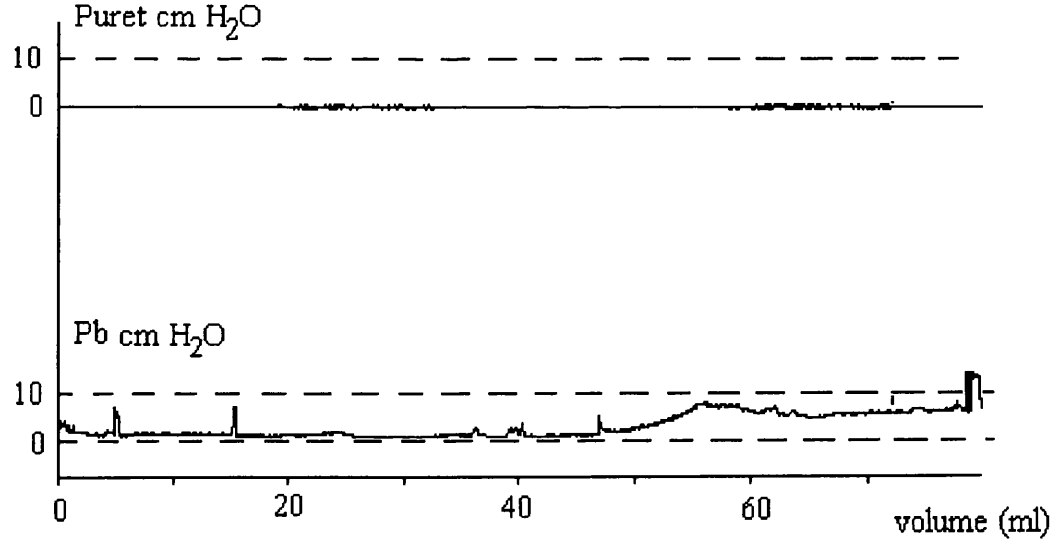


Figure 36-B - Halothane concentrations of 2%.

Bladder capacity was measured under different anaesthetic levels in 15 experiments in rabbit. The relevant results are summarised in table 5. The average bladder capacity and standard errors in these experiments were calculated and are presented in figure 37. Bladder capacity increased with anaesthetic concentration almost doubling in volume as the halothane concentration increases from 0.5% to 3%. The variation in bladder capacity as a function of the anaesthetic levels is highly significant ($p<0.0001$, Kruskal-Wallis non-parametric ANOVA test).

BLADDER CAPACITY				
Anaesthetic	Max (ml)	Range (ml)	Mean (ml)	SEM (ml)
Hyp+Diaz	60	20 - 60	43.7	3.9
0.5%H	60	20 - 60	47.7	3.8
1%H	120	40 - 120	74.3	7.1
2%H	120	50 - 120	87.3	7.7
3%H	150	50 - 150	97.3	7.7

Table 5 - Bladder capacity at a range of different anaesthetics concentrations. SEM is the standard error of mean.

Average bladder capacity at different anaesthetic levels in rabbits.

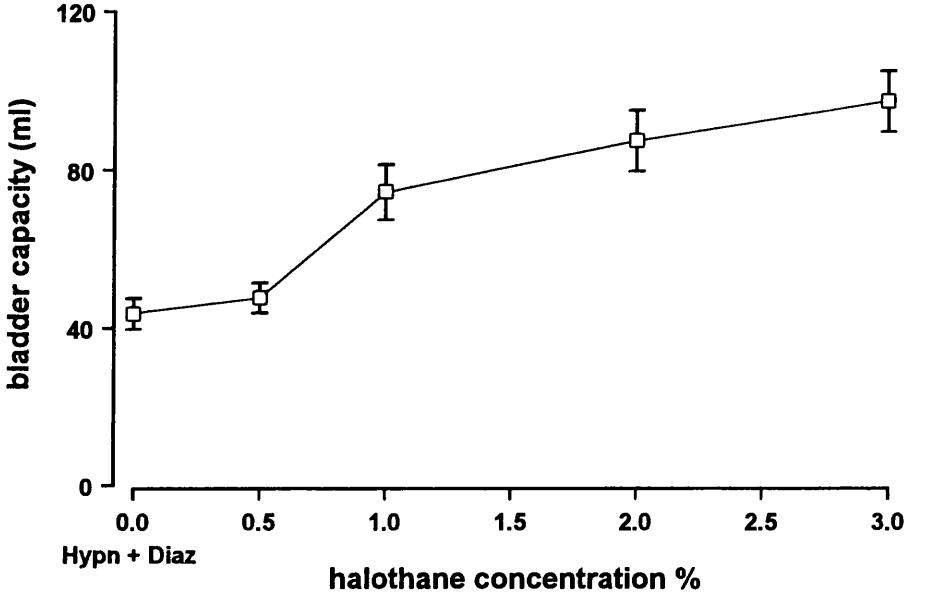


Figure 37 - Bladder capacity was measured at different anaesthetic levels in 15 experiments in rabbits. The average bladder capacity and standard erros in these experiments were calculated and are presented in the graph above. The capacity increased with the anaesthetic depth. With Halothane concentrations of 3%, bladder capacity reached more than twice the initial average capacity.

Hyp + Diaz = Anaesthesia by combination of Hypnorm and Diazepam.

The variation of pressure in the bladder and the urethra was also observed. Maximum bladder pressure, defined as the pressure in the bladder just before micturition started, was measured under different anaesthetic levels, the results are presented in table 6. There is a substantial decrease in maximum bladder pressure when the rabbit is deeply anaesthetised.

The average bladder pressure and standard errors in these experiments were calculated and are presented in figure 38. Intravesical pressure decreased by increasing the halothane concentrations. The variation of bladder pressure as a function of the anaesthetic levels is significantly greater than expected by chance ($p<0.0001$, Kruskal-Wallis non-parametric ANOVA test)

BLADDER PRESSURE				
Anaesthetic	Max (cmH_2O)	Range (cmH_2O)	Mean (cmH_2O)	SEM (cmH_2O)
Hyp+Diaz	50	20-50	33.3	4.9
0.5%H	50	20-50	33.4	9.7
1%H	40	10-40	19	5.6
2%H	30	0-30	12.3	5.4
3%H	20	0-20	8.4	3.7

Table 6 - Bladder pressure according to anaesthetic levels. Range (cm H_2O) means the range in maximum bladder pressure measured in different rabbits, probably according to the size of rabbits. SEM is the standard error of mean.

Maximum bladder pressure at several anaesthetic levels in rabbits

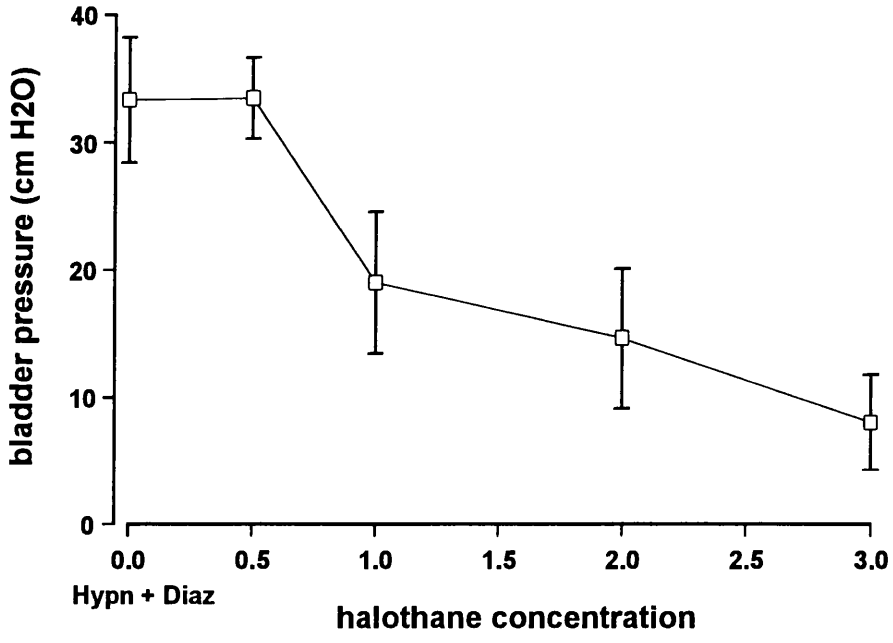


Figure 38 - Maximum bladder pressure was measured under different anaesthetic levels in 15 experiments in rabbits. The average pressure and standard error in these experiments were calculated. Bladder pressures were higher with lower anaesthetic levels. The line in the graph indicates that pressure decreased with anaesthetic depth.

Hyp + Diaz = Anaesthesia by combination of Hypnorm and Diazepam.

The maximum pressure along the urethra was also measured under different anaesthetics levels, and are presented in table 7. Although the variation of maximum urethral pressure is greater, there is only a substantial decrease in urethral response after halothane concentrations of 2%.

The mean and standard errors of these urethral pressures were calculated and are presented in figure 39. Urethral pressure generally decreased with anaesthetic depth. Analysis of variance showed that these results were significant ($p<0.01$, Kruskal-Wallis non-parametric ANOVA test).

URETHRAL PRESSURE				
Anaesthetic	Max (cmH_2O)	Range (cmH_2O)	Mean (cmH_2O)	SEM (cmH_2O)
Hyp+Diaz	35	10-35	23.8	4.3
0.5%H	30	5-30	16.2	5.5
1%H	30	10-30	16	4.7
2%H	12	0-12	5.6	2.3
3%H	4	0-4	2.3	1.2

Table 7 - Urethral pressure at different anaesthetic levels.

During sacral root stimulation, the experiments were performed with the rabbits deeply anaesthetised with 3% halothane. In these conditions no spontaneous response either from the bladder or from the urethra should be expected and therefore, any activity recorded in the bladder or urethra can safely be attributed to the stimulation.

Average urethral pressure at different anaesthetic levels in rabbits

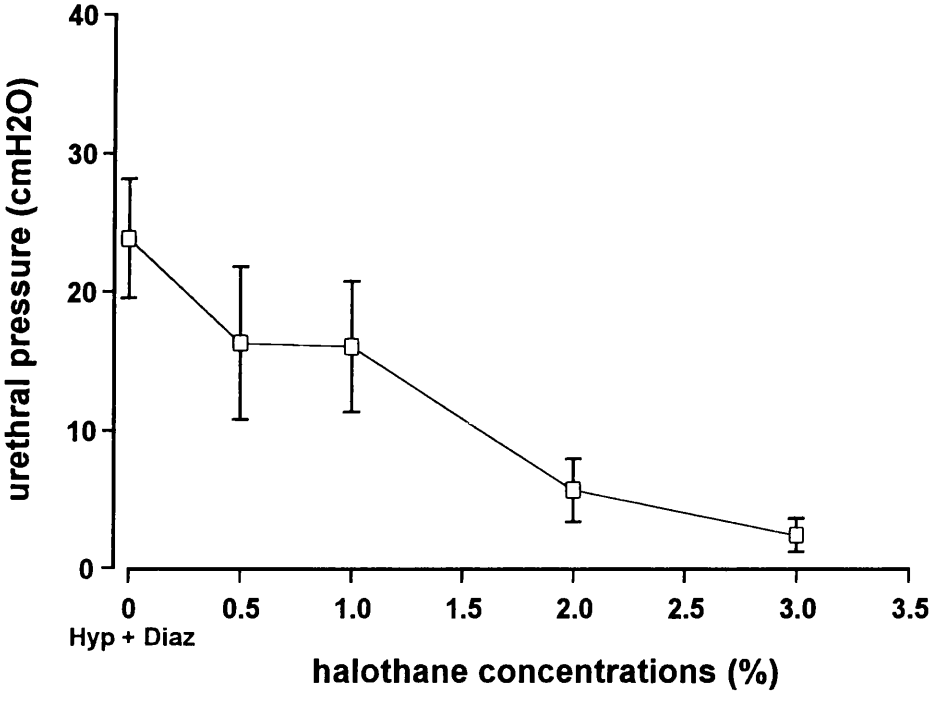


Figure 39 - The maximum pressure along the urethra was measured under different anaesthetic levels in 15 experiments in rabbits. The mean and standard errors of these urethral pressures were calculated and are presented in the graph above. Urethral pressure decreased with anaesthetic depth.

Hyp + Diaz = Anaesthesia by combination of Hypnorm and Diazepam.

6.2.4 - Effects of Rhizotomy in the Acute Experiments

Section of the spinal cord is known to cause modifications in the lower urinary tract behaviour. In humans who have suffered spinal injury, these responses are different in acute and chronic patients. In the literature, the time referred to as acute spinal cord lesion may vary anywhere from the first hours after the spinal section to the first few days. Garry et al (1957) observed the effect of acute transection of spinal cord in the lower urinary tract in a classical study carried out in decerebrate cats in Glasgow. After transection of the spinal cord at the lower thoracic level, no reflex rise in intravesical pressure was observed in response to filling of the bladder during the duration of an acute experiment. Activity in the external sphincter also disappeared but returned within 2 hours of spinal section.

The lower urinary tract behaviour is rarely investigated immediately after a spinal injury. When the patient arrives at the hospital after a spinal trauma, there are more important factors that need to be checked first, these frequently been life saving manoeuvres. However, in the experiments performed during this research, the lower urinary tract reactions were checked within one hour after laminectomy and rhizotomy. Therefore, it is necessary to investigate if the urinary tract response to the spinal division interferes with the results. Although a flaccid bladder is expected after dorsal rhizotomy, the bladder response to acute spinal shock may be either hypereflexia or areflexia. It is important to start the stimulation with a flaccid bladder, i.e., a bladder unable to contract spontaneously, so any observed response can be attributed to the stimulation.

Urodynamic studies (cystometrogram and urethral pressure profile) were performed 30 minutes after the laminectomy in 5

experiments and immediately before each stimulation period in the majority of experiments to observe bladder response prior to stimulation. Figure 40 shows an example of such CMG. In none of the experiments were spontaneous bladder contractions observed. However, these experiments do not distinguish between rhizotomy or anaesthesia as the reason for the absence of spontaneous activity .

The laminectomy was always performed in deeply anaesthetised rabbits, as was any sacral root stimulation. The results so far suggest that at this level of anaesthesia and after dorsal rhizotomy, no spontaneous response is expected from the urinary bladder. Therefore, any observed contraction must have been induced by the stimulation.

Bladder and urethral response after acute rhizotomy

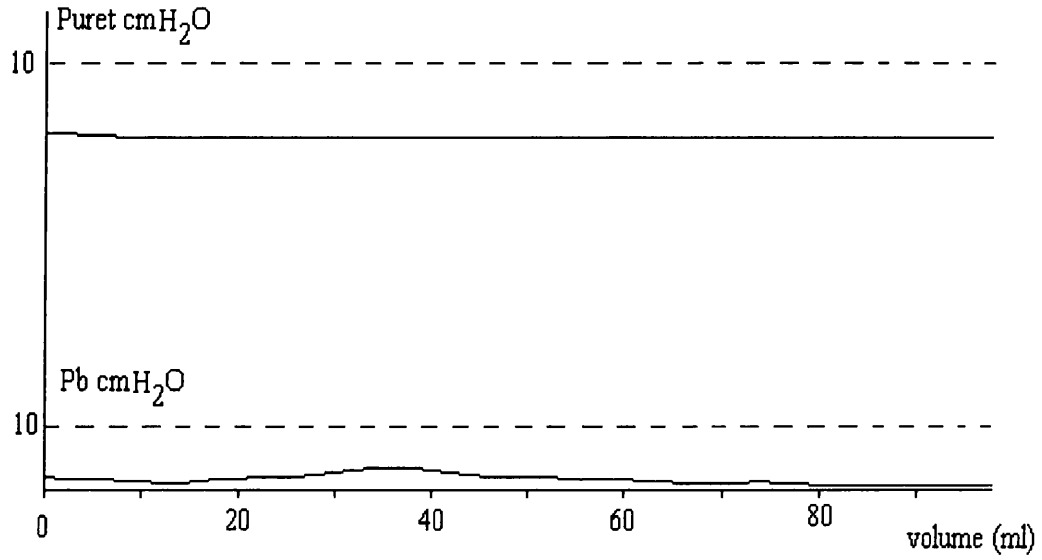


Figure 40 - Bladder and urethral responses observed thirty minutes after dorsal rhizotomy in a deeply anaesthetised male rabbit. There was no evidence of spontaneous contractions in the urethra and bladder in response to bladder filling.

Puret = urethral pressure, Pb = bladder pressure.

6.2.5 - Testing the Excitability of the Larger Motor Axons

The excitability of the larger motor axons, measured from the escape anode was tested in 15 rabbits by using the same protocol. Initially, the second ventral sacral root (S_2) was stimulated in bipolar mode. The frequency of stimulation and the pulse duration were kept constant at 20 Hz and 300 μs. The current intensity was increased progressively until skeletal muscle activity began. It was identified initially by observation of muscle movements, most frequently in the tail. This guided the insertion of fine wire electrodes which allowed recording of EMG signals. The stimulating electrodes were then switched into a tripolar configuration, which maintains the cathode current constant but splits into two anodal currents in the ratio of 9:1. Skeletal muscle contraction stopped and no EMG signal was recorded. Returning to bipolar stimulation at constant current, the EMG wave returned.

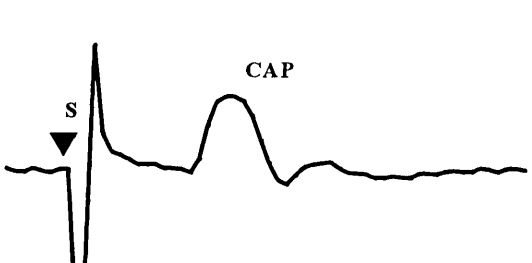
Figure 41 shows the recording from such an experiment. The compound action potentials in figure 41 are probably due to activation of motor units supplied by larger somatic axons. Measurement of the conduction velocity of these axons is not straightforward. Firstly, the conduction distance is difficult to estimate accurately and the dissection of the whole nerve is complex. Secondly, although the latency from stimulation to the beginning of an action potential can be measured accurately, this contains not only time for nerve conduction but also a neuromuscular delay and time to propagate along the muscle. In these experiments the recording electrodes were placed far from the stimulation site in the sacral root. The distance between the cathode and the recording electrode is around 55 mm. Analysis of the figure 41

shows that the time between stimulation and action potential is 1 ms. Part of this time is due to the neuromuscular junction delay.

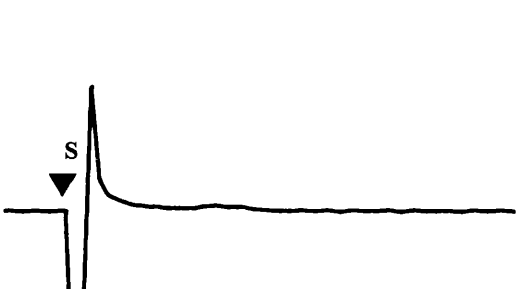
A different response is seen in another experiment, which is showing in figure 42. A similar experimental protocol to the one used before was employed. A compound action potential was set up with bipolar stimulation (A). Then, the same current intensity was applied in tripolar stimulation, keeping the cathodal current constant but dividing the anodal current as before (B). This blocks action potential propagation in the largest axons as expected. This is in line with the predicted characteristics of the electrode. However, a further increase in the current intensity during tripolar stimulation evoked a compound action potential (C). According to the model predictions, the blocking action is expected to be continuous even with higher currents. The latency of this compound action potential is the same as that the CAP evoked during bipolar stimulation. This must mean that the excitation occurs at about the same position, regardless of the conduction distance and the neuromuscular delay. Since the cathode is the most distal of the wires during bipolar stimulation the excitation in figure 42-C must also be at or near the distal end of the cuff. With the cuff set up in tripolar mode, if the excitation had occurred at the cathode, the conduction distance would be 7 mm longer causing a subsequent delay in the action potential response. But this delay did not occur. This can be explained by the presence of a virtual cathode and will be discussed later in this thesis. Figure 43 shows a diagram with the changes that may occur in the tripolar electrode with a significant increase in the current.

Average action potentials recorded during bipolar and tripolar stimulation

Bipolar



Tripolar



Bipolar

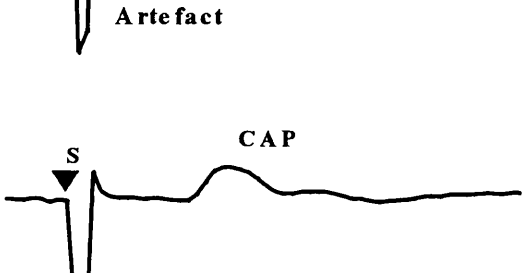


Figure 41 - Averaged action potentials recorded through a EMG needle placed at the tail of a New Zealand rabbit during bipolar and tripolar stimulation of unilateral ventral sacral root. Stimulus current: 2.2 mA, 300 μs, 20 Hz.. Compound action potential (CAP) recorded during bipolar stimulation. The same muscle response during tripolar stimulation. The stimulus parameters were kept unchanged. No action potential is recorded. Returning to bipolar stimulation, the action potential confirms muscle activity (2.6 mA).

Each trace is the mean of 256 repetitions.

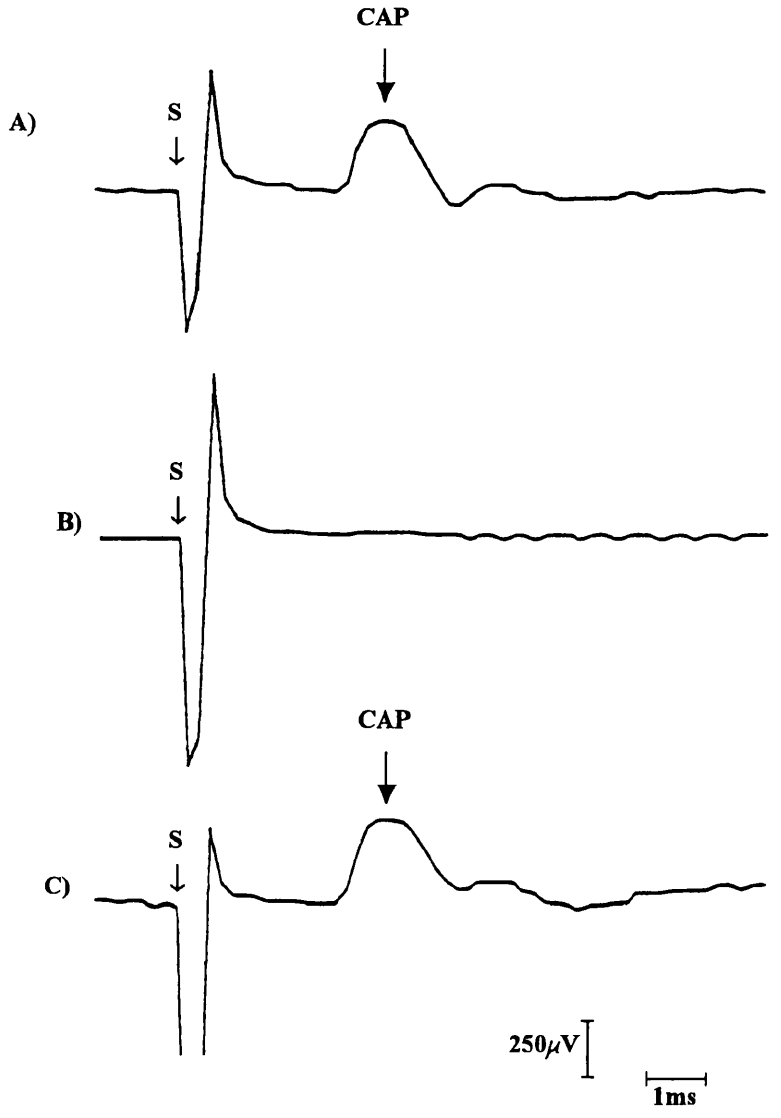


Figure 42 - Averaged action potentials recorded during bipolar and tripolar stimulation. Each trace is the average of 256 repetitions

A - Compound action potential (CAP) recorded through a EMG needle placed at the tail of a New Zealand rabbit during bipolar stimulation of unilateral sacral root.

B - The same muscle response during tripolar stimulation. The stimulus parameters were kept unchanged. No action potential was recorded.

C - Still in tripolar stimulation, the current intensity was increased and an action potential was recorded.

S = start of stimulation

Stimulation in a cuff electrode

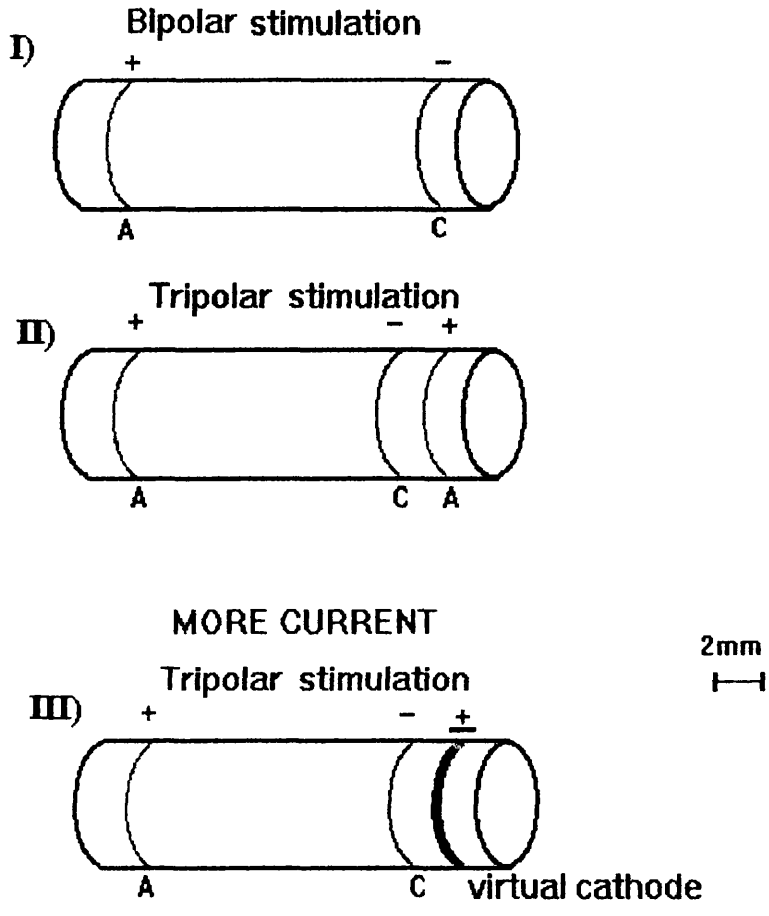


Figure 43 - This diagram illustrates the several possible ways in which stimulation may occur with a cuff electrode

I: Bipolar cuff electrode: the excitation occurs at the cathode (- C).

II: Tripolar cuff electrode: excitation still occurs in the cathode. The same cathode current used in **I** is divided in two different anodal currents (+ A).

III: Virtual cathode at the end of a tripolar cuff electrode. If the current that flows to the anode is large enough, the excitation can occur near to the end of the cuff. This new point of excitation is knowing as a virtual cathode.

The range of currents with which skeletal muscle contractions were activated by stimulating in bipolar mode and in which contractions were stopped with the same current during tripolar stimulation are shown in the table below. These data are taken from the experiments in 15 rabbits

skeletal muscle contraction		Current (μA)
Bipolar	Tripolar	
yes	no	60
yes	no	130
yes	no	400
yes	no	500
yes	no	700
yes	no	700
yes	no	800
yes	no	800
yes	no	1000
yes	no	2000
yes	no	2000
yes	no	2000
yes	no	2100
yes	no	2200
yes	no	2200

Table 8 - Experiments showing skeletal muscle response to bipolar and tripolar stimulation. Data sorted by the minimum current nedded to activate the tail muscle.

During tripolar stimulation, the contractions could have been stopped by blocking the propagation of action potential in skeletomotor axons or because the current used was not sufficient to initiate action potentials. So, more analyses were made comparing the experimental results to the model predictions, to see if the anodal block of the skeletomotor axons occurred.

The Threshold Currents

The intensity of anodal currents needed to activate the skeletomotor axons during bipolar stimulation and to block propagation during tripolar stimulation was investigated at the escape end of the cuff electrode. In 15 experiments, the minimum current needed for achieving skeletal muscle contractions varied between 60μA to 2200 μA during bipolar stimulation. The range of currents is related to stimulation of roots of different diameters requiring different intensities of currents, accidental nerve damage during surgical manipulation, reduced nerve excitability and current loss into surrounding tissues

Figure 44 shows these currents and the predicted minimum blocking. In eleven out of fifteen experiments the measured current was above the minimum current predicted. These results are similar to those reported in chapter 5. However, in 4 cases currents smaller than the ones predicted stopped the muscle contractions. This might be due to reduced excitability making these axons more susceptible to block.

Minimum current intensity to block the larger fibres at the escape end of the cuff electrode

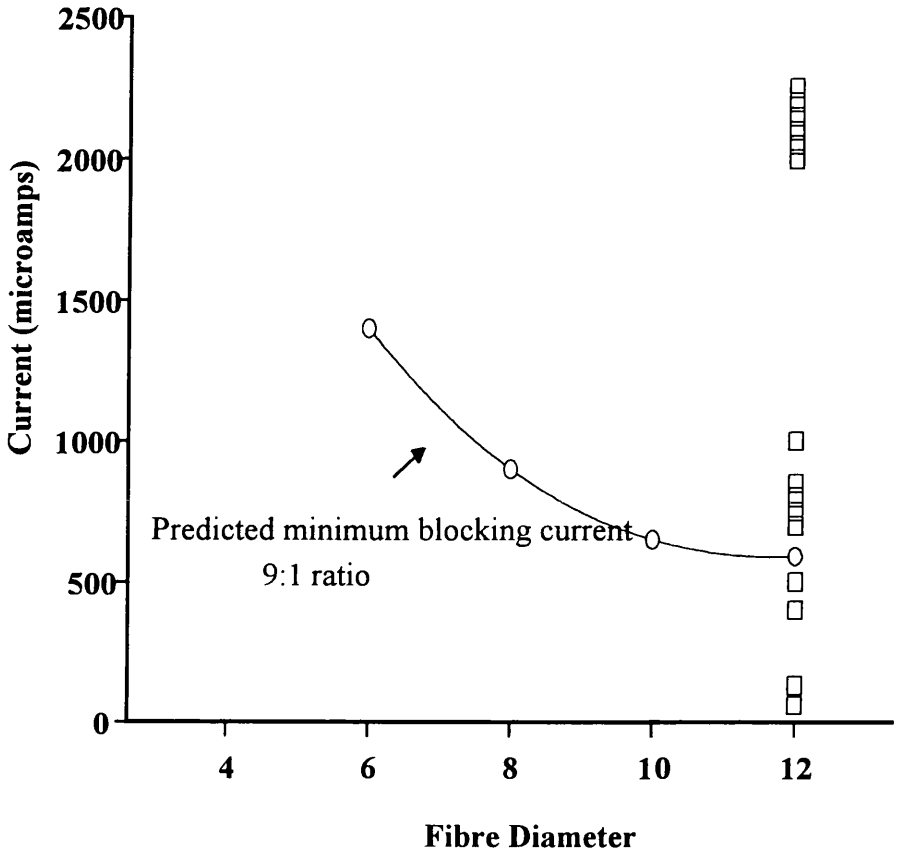


Figure 44 - The minimum current intensities needed to block the larger skeletomotor axons at the escape end of the cuff electrode during tripolar stimulation of the ventral sacral root in rabbits are compared to the Fitzpatrick model predictions. Current intensities to achieve blocking were over the minimum predicted currents in 11 experiments. However, blocking was achieved with less current than predicted in 4 experiments.

Key: 0 - model predictions

□ - experimental results

6.2.6 - Testing the Selective Activation of the Small Autonomic Axons at the Escape End

The previous experiments studied the behaviour of larger somatic axons using the EMG as an indicator of activation in the cuff. Any stimulation of smaller somatic or autonomic axons is unlikely to be observed.

The experiments were modified to investigate if smaller axons are activated during tripolar stimulation. Once again, indirect measurements such as bladder pressure as an indicator of activation of small autonomic axons were used. The pressure in the urethral sphincter was used as an indicator of activation of smaller somatic axons. Bladder and urethral pressure were recorded by conventional urodynamic methods as described in the methods section.

Initially, the second ventral sacral root (S_2) was stimulated in bipolar mode. The frequency of stimulation and the pulse duration were also kept constant at 20 Hz and 300 μs. The current intensity was increased progressively until skeletal muscle activity began, most frequently in the tail. The currents were increased further until bladder and urethral sphincter contractions began. The current thresholds for skeletal muscle, bladder and urethral sphincter excitation were measured. The electrodes were then switched into the tripolar configuration and the current threshold measurements were repeated.

The key aim of these experiments is to observe that selective activation of autonomic motor fibres is possible during tripolar stimulation, such that bladder contraction can be stimulated at lower current thresholds than skeletal muscle.

The responses of the bladder, external urethral sphincter and tail muscle to bipolar and to tripolar stimulation were observed in 28 experiments.

The results of these threshold measurements are given in table 9. The variation in threshold currents between different experiments make comparison between different experiments very difficult. The opinion of the Statistical Advisory Service of The University of Glasgow was sought and followed. Mr McLaren advised that the data be analysed as a series of paired observations and that the analysis should be done using the statistics of binomial distribution.

Each pair of observation gives rise to a single observation of which type of stimulation has the lower current threshold. For example, when the current thresholds for skeletal and bladder contractions are compared in 27 experiments, it is seen that skeletal muscle thresholds are lower in each case. Using the null hypothesis that there is no difference between muscle and bladder thresholds, the binomial probability of the observed sequence having occurred by chance was calculated. The probability is $p < 0.0001$ and so the null hypothesis can be rejected with confidence and it is safe to conclude that with bipolar stimulation the skeletal muscle thresholds are significantly lower than bladder thresholds.

A similar analysis was performed to compare the significance of all the possible combinations of tissue thresholds in both stimulation modes. The results are summarised in table 10. This analysis confirms that the recruitment sequence seen during bipolar stimulation is reversed during tripolar stimulation for each combination of structures. In particular, with tripolar stimulation the bladder contractions are initiated at lower currents than sphincter contractions ($p < 0.0001$).

Table 9 - Summary of the results from 28 experiments carried out in rabbits to test the possibility of selective activation of the small autonomic motor axons at the escape end of a tripolar electrode. The data are sorted by the minimum current to activate the tail muscle. Currents are expressed in milliamperes.

During the first 15 experiment (upper section), no sphincter response was measured. The aims of these experiments were to observe the relationship between skeletal muscle and bladder thresholds in bipolar and tripolar mode. The stimulation started in bipolar mode. Tail muscle contracts with lower currents than the bladder. Then, the stimulation was changed to tripolar mode, and bladder thresholds were measured again.

During the second group of 13 experiments, tail muscle, bladder and sphincter thresholds were observed during bipolar and tripolar stimulation. The stimulation started in bipolar mode, and the current intensity increased until the thresholds for skeletal (tail and sphincter) and smooth (bladder) muscles were measured. Then, the stimulation was changed to tripolar mode and the thresholds measurements were repeated.

Summary of Results

tail bipolar μA	bladder bipolar μA	bladder tripolar μA
60	280	240
130	350	300
400	500	400
500	600	600
700	2500	2500
700	2500	2500
800	1200	1100
800	1200	1000
1000	4800	3000
2000	2500	2400
2000	2500	2400
2000	3800	2300
2100	4000	2400
2200	4000	2200
2200	4000	2200

In the experiments above the sphincter response was not recorded.

tail BIP	tail TRIP	sphincter BIP	sphincter TRIP	bladder BIP	bladder TRIP
40	1000	400	800	600	600
40	-	1000	-	2000	1000
200	680	400	560	600	400
200	-	400	-	600	400
200	-	800	-	1600	500
280	1100	480	600	4200	500
300	-	400	-	600	320
300	640	440	2400	600	320
500	4400	1000	4000	2000	1200
600	1000	1000	960	1500	520
1000	4400	3000	4200	4000	1800
1500	4400	2400	4200	4800	1200
1500	4400	2400	4200	4800	1120

Complete experiments, where tail muscle, bladder and urethral sphincter were activated during bipolar or tripolar stimulation. However, in 4 experiments the range of current available (5mA) was not enough to activate the urethral sphincter and the tail during tripolar stimulation.

Table 9

Binomial Variation of possible combinations of responses to stimulation.

Possible Combinations	First to contract Bipolar Mode	First to contract Tripolar Mode
smooth x skeletal	skeletal (n = 27) **p < 0.0001**	smooth (n = 27) **p < 0.0001**
bladder x sphincter	sphincter (n =13) **p < 0.0001**	bladder (n = 13) **p < 0.0001**
bladder x tail	tail (n = 27) **p < 0.0001**	bladder (n = 27) **p < 0.0001**
tail x sphincter	tail (n=13) **p < 0.0001**	sphincter (n = 9) **p < 0.01**

Table 10 - Stimulation of a specific ventral sacral root (S_2) activates the tail muscle, the urethral external sphincter and the urinary bladder in rabbits. However, their current thresholds are different, therefore each one is activated in turn. It is important to know if there is any selectivity between them according to the stimulation process used. The response to bipolar and tripolar stimulation was observed in order to test the muscle that responds first, a statistically compared by using the binomial distribution for each possible combination. The results are extremely significant, confirming that bladder is activate first using tripolar stimulation.

n represents the number of successful experiments.

6.2.7 - A more detailed study of the stimulation response on the different pairs.

The previous section shows clearly that it is possible to reverse the expected bipolar recruitment sequence using tripolar stimulation. Although it is desirable to find the range of currents to apply in future experiments, the great variation in current needed for selective activation made the specification of a suitable current range difficult. Statistical advice was taken (McLaren, Statistical Advisory Service). Since the distribution of current intensities is not normal, the application of parametric tests and calculation of mean, standard deviation and confidence interval are inappropriate. The statistically safest way is to analyse the ranges of current as pairs of observations, using the absolute values of current intensities. These results are presented in the next sections.

6.2.7.1 - Bladder Thresholds during Bipolar and Tripolar Stimulation:

The current thresholds for bladder contractions were measured during bipolar and tripolar experiments.

During tripolar stimulation, these thresholds were lower than during bipolar stimulation in 23 out of 28 experiments. In the other 5 experiments there was no difference in thresholds. Details of the currents used can be found at table 9, page 126.

Significantly less current was needed to activate the bladder with tripolar stimulation. This difference can be seen clearly when the current thresholds are compared in figure 45. The minimum currents needed to

contract the bladder during tripolar stimulation are plotted as a function of the minimum currents to contract the bladder in bipolar stimulation. If the currents intensities were equal, they would fit a line at 45° (broken line). Bipolar currents and tripolar currents were the same in five cases. Since all the other points are under this line, tripolar currents are lower than bipolar current ($y<x$). This graphical representation supports the statistical tests ($p<0.0001$, binomial distribution) as described earlier in section 6.2.6.

The ability to stimulate the bladder with a lower current intensity is very important in preventing the unwanted effects of higher current intensities on the nerve and neighbouring tissues.

Currents thresholds for bladder activation during bipolar and tripolar stimulation.

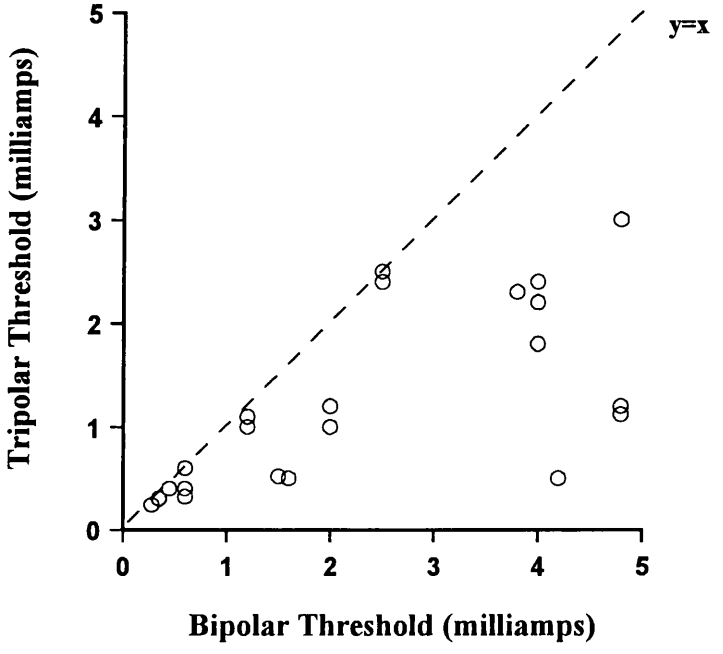


Figure 45 - Minimum current for bladder activation during bipolar stimulation and tripolar stimulation in 28 rabbits.

6.2.7.2 - Bladder and Skeletal Muscle Thresholds during Bipolar and Tripolar Stimulation

The currents thresholds for bladder and skeletal muscle (tail muscle) contractions were measured during bipolar stimulation and tripolar stimulation. The results are presented in table 9, page 126.

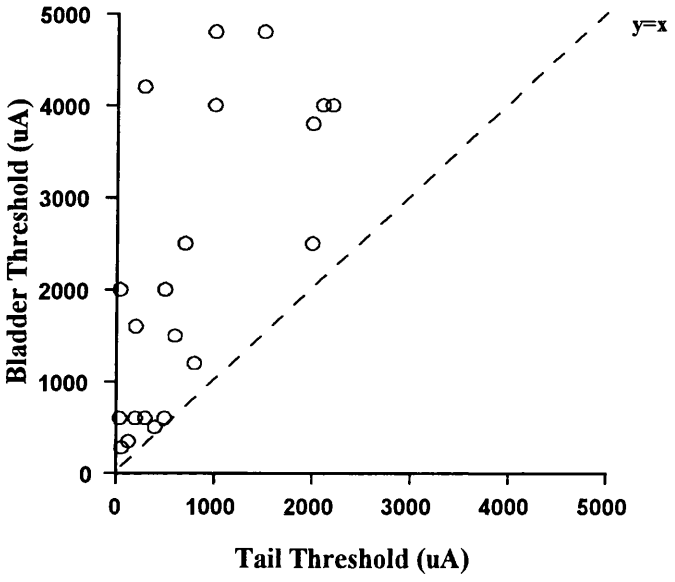
During bipolar stimulation a higher current intensity was needed to activate the bladder in 28 experiments. These results confirm the expected recruitment sequence with bipolar stimulation.

During tripolar stimulation, tail contractions were observed in 9 experiments. This small number of observations is due to the relative limited maximum current delivered by the stimulator, 5mA. In all nine experiments, bladder contractions were elicited with lower current thresholds than tail contractions.

These differences can be clearly seen in figure 46. During bipolar stimulation, bladder thresholds are bigger than tail thresholds. During tripolar stimulation, bladder thresholds are lower than tail thresholds.

Currents thresholds to activate bladder and tail muscle during sacral root stimulation

Bipolar



Tripolar

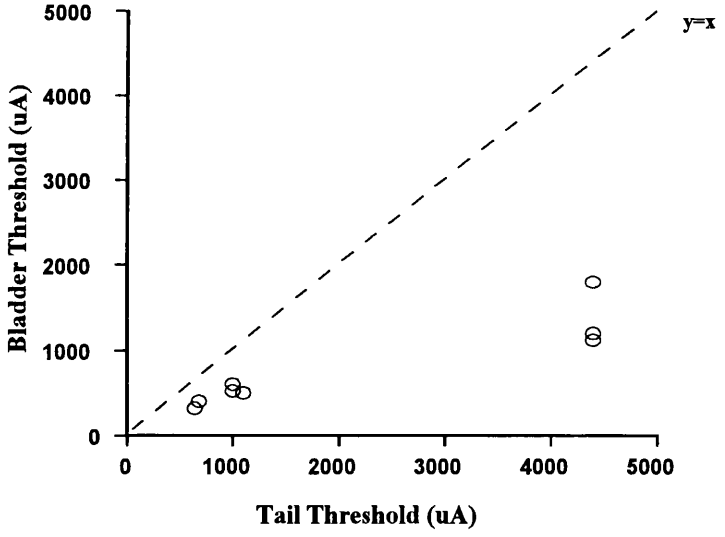


Figure 46 - Minimum current for activation of the skeletal muscle from the tail and the bladder detrusor with bipolar and tripolar stimulation.

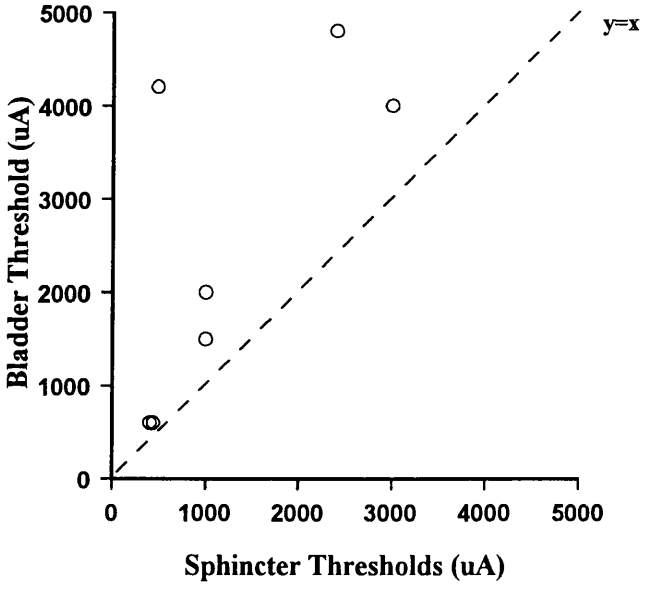
6.2.7.3 - Bladder and urethral sphincter thresholds

The currents thresholds for activation of bladder and urethral external sphincter were measured during bipolar and tripolar stimulation. The results are presented in table 9 on page 126.

As described in section 6.2.6, urethral sphincter contraction always occurred at a lower current threshold than bladder contraction during bipolar stimulation. These data also confirmed the expected recruitment sequence with bipolar stimulation. During tripolar stimulation, bladder contractions always occurred at a lower current threshold than sphincter contraction. However, sphincter contractions were only recorded in 9 experiments, probably due to limited maximum current delivered by the stimulator, 5mA. Figure 47 shows sphincter thresholds as a function of bladder thresholds during bipolar and tripolar stimulation. The bipolar recruitment sequence is reversed.

Currents thresholds for bladder and sphincter activation

Bipolar



Tripolar

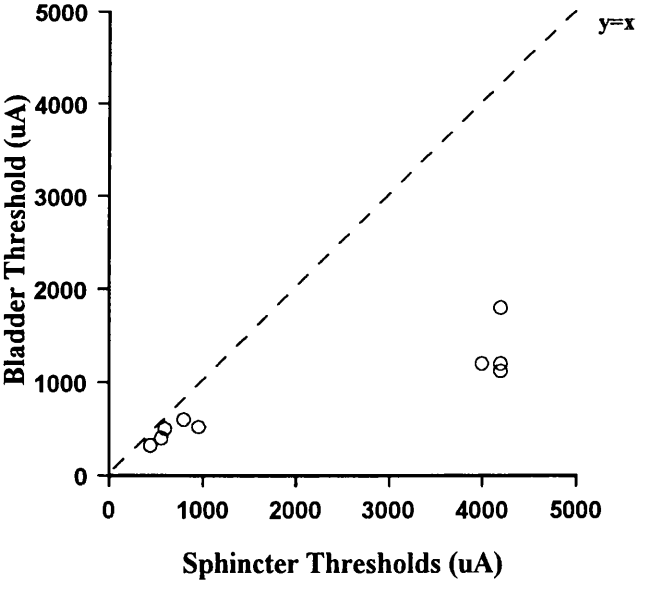


Figure 47 - Minimum current for bladder and sphincter activation during bipolar and tripolar stimulation in 9 experiments.

6.2.7.4 - Tail muscle and urethral sphincter thresholds

The previous results showed that with tripolar stimulation it is possible to activate selectively small autonomic motor axons and block the larger motor axons. However, the somatic motor axons contained in the ventral sacral roots vary in size. The skeletomotor axons which innervate the external urethral sphincter are usually smaller than the skeletomotor axons that innervate skeletal muscles such as the tail muscle. Therefore, it is useful to test if the selective blocking action is capable of even greater selectivity, i.e. independent control of larger axons which supply the tail muscle and intermediate axons which supplies the urethral sphincter. This information may be useful in preventing unwanted contractions of neighbouring tissues during sphincter stimulation.

In order to test this selective blocking, the current thresholds for activation of tail muscle and external urethral sphincter were measured during tripolar stimulation. The results are presented in table 9, page 126. The same protocol from the previous section was used to analysed these data.

During bipolar stimulation, the tail muscle and the urethral external sphincter were activated in 13 experiments. Tail contractions always occurred at lower current intensities than urethral sphincter contractions.

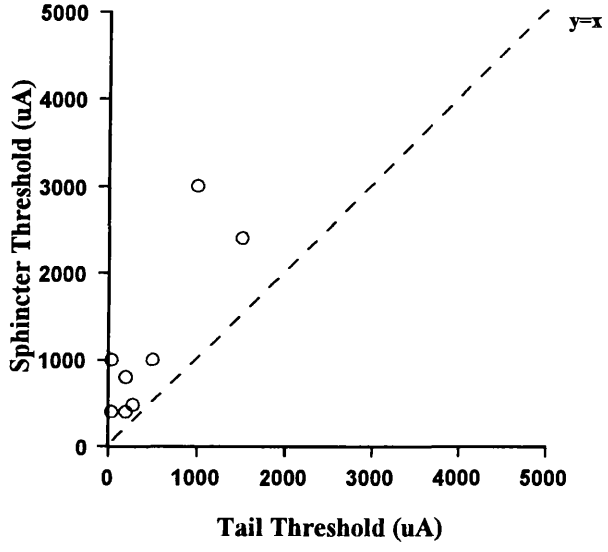
During tripolar stimulation, the tail muscle and the urethral external sphincter were activated in 9 experiments, the urethral sphincter contractions preceding tail contractions in 8 experiments. These results are clearly seen in figure 48.

However, as seen in section 6.26 , using the null hypothesis that there is no difference between tail muscle and urethral sphincter

thresholds, the binomial probability of this observed frequency of circumstances having occurred by chance was calculated. The probability is $p<0.01$. It is safe to assume that during tripolar stimulation the sphincter thresholds are significantly lower than the tail thresholds (table 10, page 127).

Currents thresholds to activate tail muscle and urethral external sphincter during sacral root stimulation

Bipolar



Tripolar

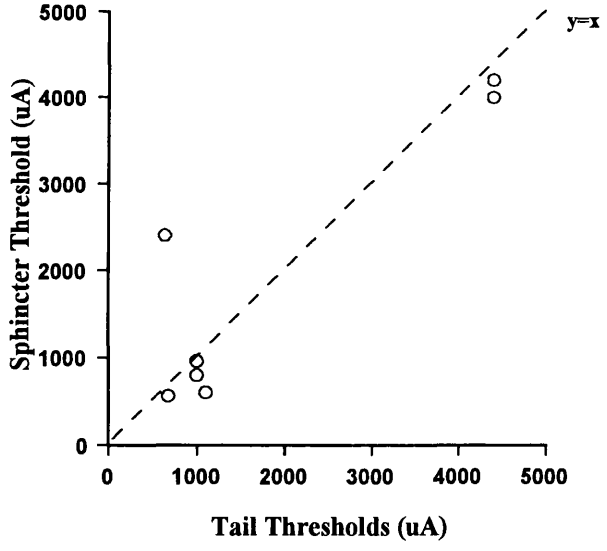


Figure 48 - Currents thresholds for activation of the skeletal muscle of the tail and the external urethral sphincter with bipolar and tripolar stimulation.

6.2.8 - Urodynamic Tests

The effect of sacral root stimulation on the bladder and urethra was measured by urodynamic evaluation. Measurements of intravesical pressure were used to identify the activation of autonomic axons supplying the bladder and measurements of intraurethral pressure to observe activation of axons supplying the external sphincter.

The rabbit urethra was catheterised with a 7Fr 30 cm triple lumen urodynamic catheter (Cook Urological^{R}). The bladder was slowly filled with warmed physiological saline, at a constant rate by an infusion pump. The intravesical pressure and the intraurethral pressure were recorded by connecting the catheter to a Elcomatic EM 751 pressure transducer which was connected to a Neurolog NL 108 pressure amplifier.

The sacral root stimulation was carried out in deeply anaesthetised rabbits and after a lumbosacral rhizotomy. As seen in section 6.2.3, page 104 and 6.2.4, page 113, in these circumstances, no spontaneous response is expected from the urinary bladder and external sphincter. Therefore, any observed contraction must have been induced by the stimulation.

Each train of stimulation pulses last 3 seconds, with a rest of 17 seconds between stimulation. The frequency of stimulation was 20 Hz and the pulse width was 300μs.

Response to bipolar stimulation:

Figure 49 shows the results of urodynamic evaluation in a typical experiment. Bladder and urethral sphincter pressures were recorded by conventional urodynamic methods, as described before. The experiment started with bipolar stimulation of ventral sacral root and the current intensity was increased progressively until a response in the lower urinary tract was observed. Initially, with bipolar stimulation at 0.4 mA, the external sphincter contracted and a clear increase in pressure is seen. With a further increase in the current to 0.6 mA, the bladder was also activated. This is the expected recruitment sequence in bipolar stimulation, where the larger somatic axons are activated at lower thresholds. As the current intensities are reduced during the next three periods of stimulation, the bladder contractions are reduced. During each period of stimulation the sphincter pressure was greater than the bladder pressure.

After a rest period of 20 minutes, the experiment was repeated to test the reproducibility of bladder and sphincter thresholds measurements. The results are presented in figure 50. The bipolar stimulation started with current intensities at 0,4mA and a sphincter contraction was observed. No bladder response was seen until the current intensity reached 0.7mA, when a strong bladder contraction elevates the pressure up to 40cm H_2O. At this point, the bladder pressure was greater than the urethral pressure and micturition occurred. As the current was reduced to 0.5mA, bladder contractions stopped.

Effect of Bipolar Stimulation in the Bladder and the Urethra

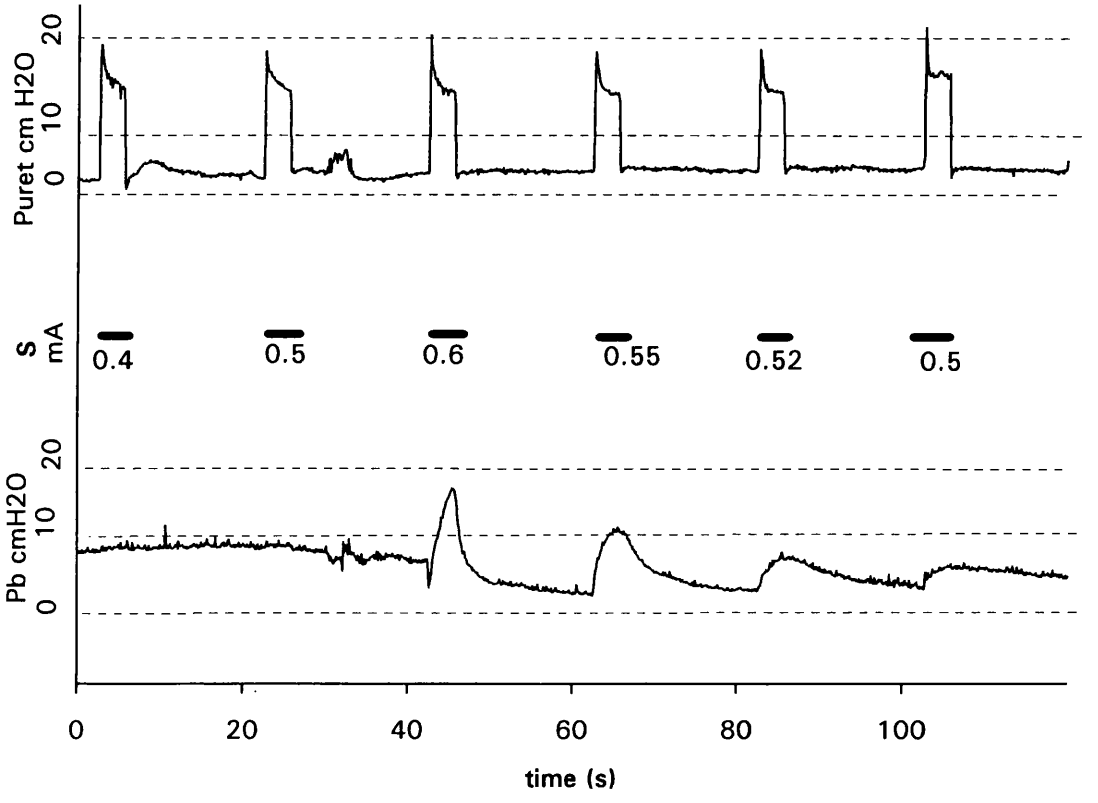


Figure 49 - Intraurethral (Puret) and intravesical (Pb) pressure responses to unilateral bipolar stimulation of the second anterior sacral root in rabbits. Each response was obtained by stimulating the root for approximate 3s, with a rest interval of 17s between stimulation. Urethral activation started at 0.4mA. Further increase in current intensity to 0.6mA started bladder contractions. The period during the stimulation had been applied (S) is indicated with traces (–). The numbers over are the currents intensities applied during each stimulation period.

Effect of Bipolar Stimulation in the Bladder and the Urethra

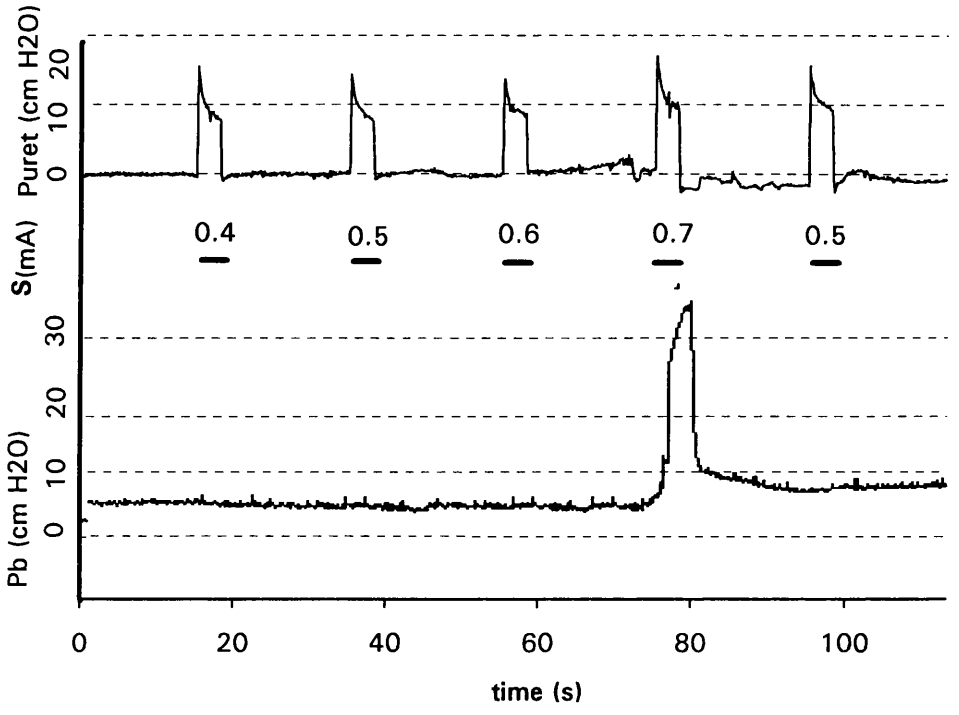


Figure 50 - These results were obtained in sequence to the ones presented in figure 49. The anterior sacral root is stimulated in bipolar mode for 3s, with a rest of 17s between periods of stimulation. The record starts at the current threshold which produced urethral activation, 0.4mA. The current was increased further still, up to 0.7mA, which produced a stronger bladder contraction. When the current intensity was lowered, the bladder contractions stopped.

The record was low pass filtered to remove movement artefacts.

Response to tripolar stimulation

The same experimental protocol was repeated to investigate the response to tripolar stimulation, except that the stimulation was tripolar with an anodal ratio 9:1. Tripolar stimulation started 20 minutes after i the experiment traces in figure 50 were recorded. The stimulation parameters were as before: pulse duration of 300μ, the stimulation pulses were delivered for 3s, followed by a rest period of 13s, at a frequency of 20Hz.

The current intensity was increased until a bladder or sphincter response was observed. At 0.32 mA bladder contractions started to be recorded, but no significant activity was seen at the external urethral sphincter. These results are presented in figure 51. Increasing the current intensity to 0.35mA elicited another bladder contraction. Decreasing the current intensity to sub-thresholds levels - below 0.3mA, stopped bladder contractions.

Figure 52 shows the sphincter being activated during tripolar stimulation. This experiment occurred in sequence with the one described above. After a 20 minutes interval, the stimulation restarted at 1 mA. Bladder contractions were recorded. A further increase in current intensity to 2.4mA also produced sphincter contractions. However, bladder pressures were always greater than sphincter pressures during tripolar stimulation.

The bipolar recruitment sequence was reversed since tripolar stimulation with current intensities below 1mA activated solely the smaller autonomic axons which innervate the bladder whilst selectively blocking the larger axons that innervate the urethral sphincter. These last responses, however, are not consistent with the model predictions.

Effect of Tripolar Stimulation in the Bladder and the Urethra

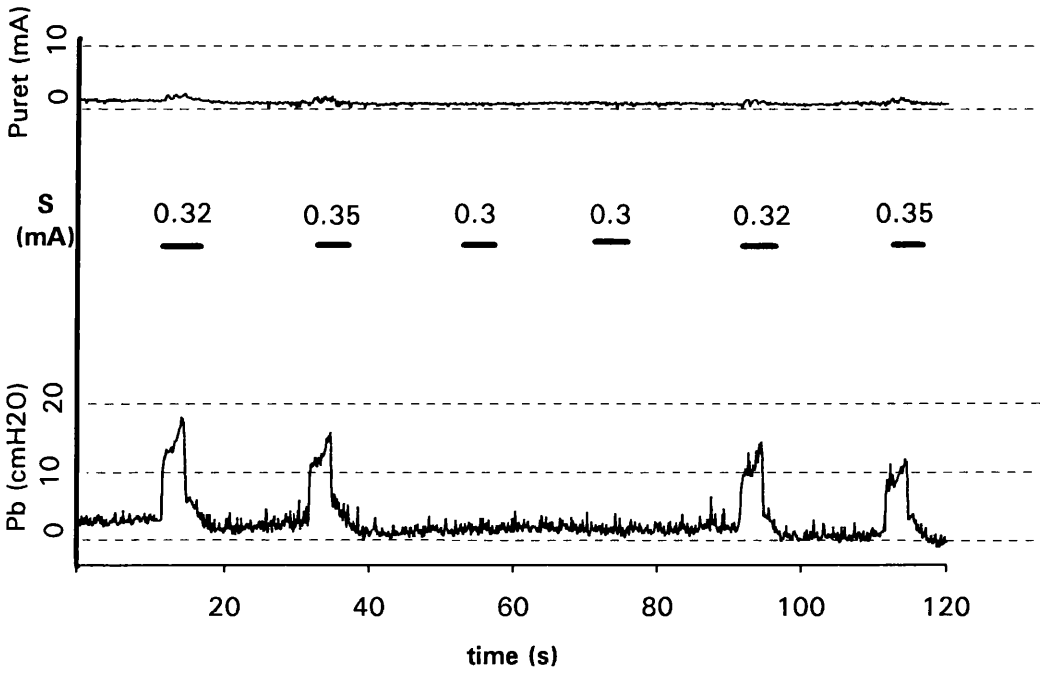


Figure 51 - Intraurethral (Puret) and intravesical (Pb) pressure responses to unilateral tripolar stimulation of the second anterior sacral root in rabbits. Each response was obtained by stimulating the root for approximate 3s each time, with a rest interval of 17s between stimulation, at a frequency of 20Hz and pulse duration 300μs. The period during which the stimulation had been applied (S) is indicated with traces (–).

Effect of Tripolar Stimulation in the Bladder and the Urethra

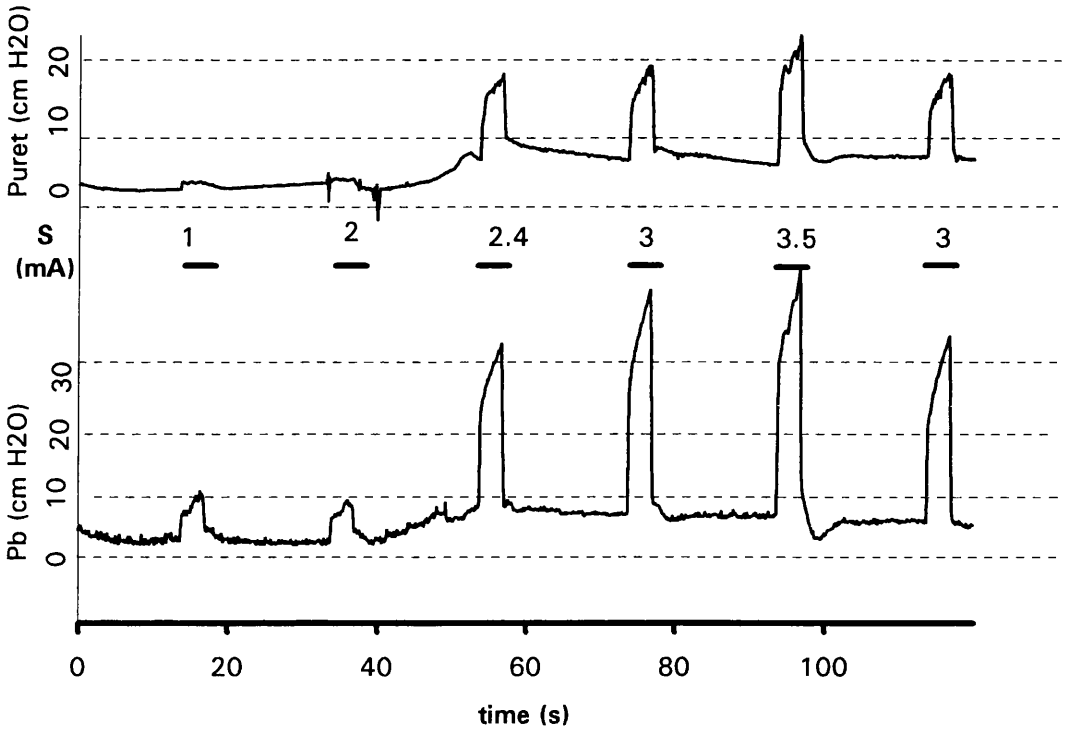


Figure 52 - These results were obtained in sequence to those presented in figure 51. The same stimulation parameters are used except for the current intensity. Bladder responses (Pb) occur with lower currents than urethral response (Puret). But when current intensity was increased further still (2.4mA) urethral contractions were recorded. Bladder pressures achieved in response to tripolar stimulation were higher than the urethral pressure.

tripolar stimulation, but can be explained in terms of excitation of a virtual cathode at the ends of the cuff due to the high current intensity. This will be discussed later.

The bladder and the urethral responses can be regulated by the current intensity. The response seems to be greater with stronger current intensities.

A simple experiment is shown in figures 53 and 54.

In figure 53, tripolar stimulation started at 2mA, which is just below the sphincter threshold. Bladder pressure rises during each period of stimulation but no activity is seem from the urethra. A further increase in current intensity to 2.4mA exceeds sphincter thresholds and results in a rise in urethral pressure. The current was increased up to the maximum allowed by the stimulator. Bladder and urethral sphincter continue to contract in response to each period of stimulation. No changes are observed until 4.5mA, when a further increase in urethral pressure appears.

The current was then reduced, as shown in figure 54. When the current is reduced below thresholds for sphincter stimulation, the urethral response stopped. At 1mA, the bladder was only weakly activated. Further increase in current intensity restores stronger bladder contractions.

Increasing the Current Intensity

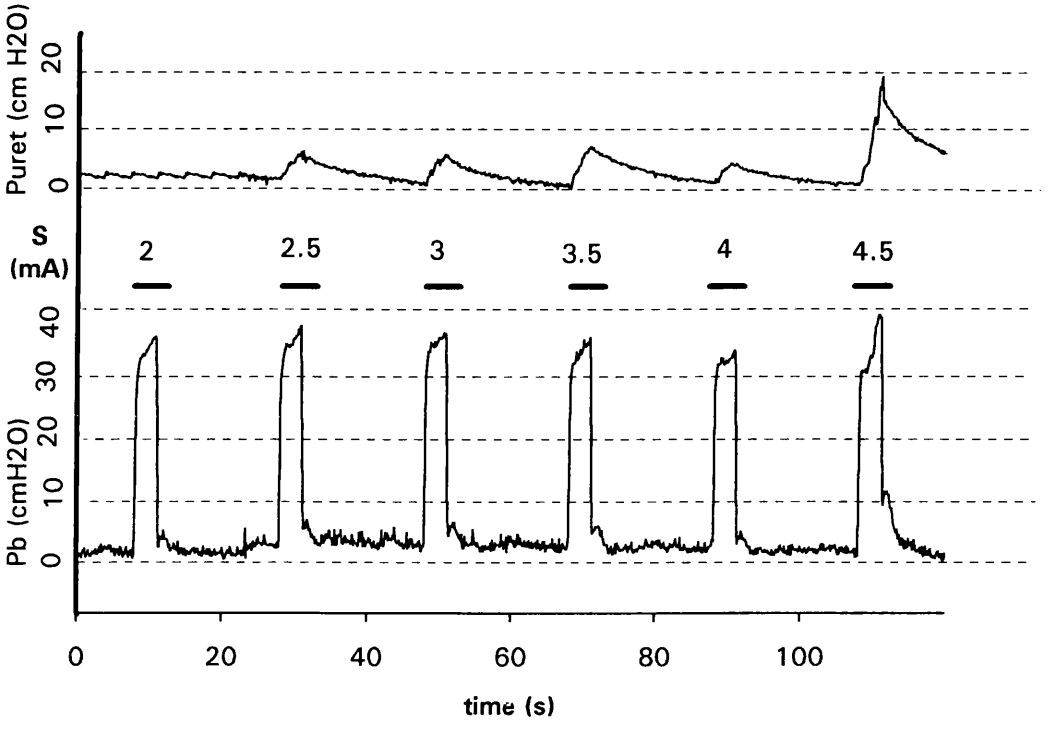


Figure 53 - Effects of increasing the current intensity on bladder and sphincter response during tripolar stimulation. Stimulation starts with 2mA. This current intensity is above bladder threshold but just below the minimum current needed to activate the urethral sphincter in figure 52. Strong bladder contractions are recorded. Further increase in current intensity activate the external sphincter. Urethral pressures increased sharply when the current was increased from 4 to 4.5mA.

Effects of Decreasing the Current Intensity

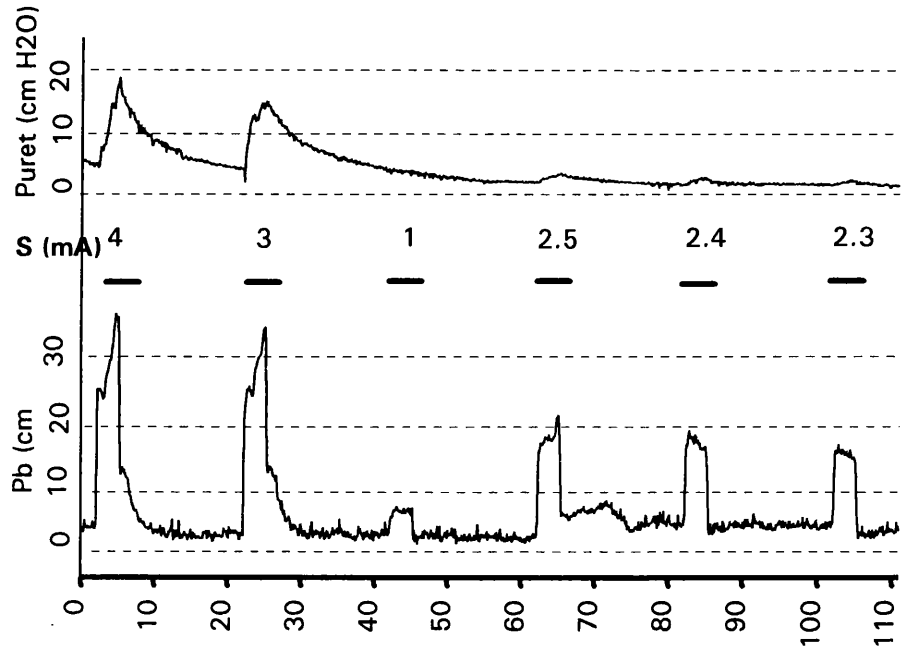


Figure 54 - These results are obtained in sequence to the ones from figure 53. The current intensity was reduced. At 1mA, no sphincter response is seen, and bladder response clearly reduced. Further increase in current intensity restored bladder response.

General results: the responses of tail, bladder and urethral external sphincter to bipolar and tripolar stimulation.

Bipolar Stimulation

In all experiments using bipolar stimulation, the skeletal muscle axons from the tail muscle were activated at the lowest current threshold. The thresholds for activation of the external sphincter were at least 50% higher. The thresholds for activation of the bladder were higher still, at least 200% greater. These results confirm the expected recruitment sequence with bipolar stimulation.

The current thresholds for activation of tail skeletal muscle contractions with bipolar stimulation ranged from 0.04 mA to 2.2 mA.

The currents for activation of the urethral sphincter ranged from 0.4mA to 3 mA. The thresholds for activation of the bladder ranged from 0.28 mA to 4.8 mA.

The ratio of these current thresholds is presented in table 11. The current thresholds for activation of the bladder and the external urethral sphincter were presented as multiples of the current thresholds for activation of the larger skeletomotor axons. With bipolar stimulation, the current thresholds for activation of the urethral sphincter were between 1.5 to 3 times greater than those for activation of the tail muscle except in experiment 5, where it is much greater. The current thresholds for activation of the bladder were between 2 to 4 times greater than those of the tail muscle except in 2 cases. In experiments 1 and 5 the current thresholds for activation of the bladder were 15 times bigger. In experiment 1 the current threshold for activation of the tail muscle was within the expected range, but bladder activation needed a much larger current. Whilst in experiment 5 the current to stimulate the

bladder was similar to other experiments, the current for activation of the tail was surprisingly low.

Tripolar Stimulation

Less current was needed to activate the bladder in tripolar stimulation. In addition, the recruitment sequence seen in bipolar stimulation is reversed with the bladder excited at lower current intensities than the skeletal muscle. With tripolar stimulation, the current thresholds for activation of the bladder ranged from 0.24 mA to 3 mA. The current thresholds for activation of the urethral sphincter were between 0.56 and 4.2 mA and the thresholds for activation of the tail skeletal muscle were between 0.64 to 4.4 mA.

The ratio of the current thresholds used during tripolar stimulation is also presented in table 11. The thresholds were normalised based in the tail threshold, i.e., the current thresholds for activation of the bladder and the external urethral sphincter were presented as multiples of the current thresholds for activation of the larger skeletomotor axons. With tripolar stimulation, current thresholds for bladder activation were between 0.27 to 0.6 times the current thresholds to tail muscle activation. The current thresholds for activation of the urethral sphincter were also smaller, between 0.55 to 0.96 times the current thresholds for tail muscle activation.

The inverse recruitment sequence with tripolar stimulation is shown in figure 55.

Bipolar Mode

Experiment	Tail	Urethral sphincter	Bladder
1	1	1.71	15
2	1	1.6	3.2
3	1	3	4
4	1	2	4
5	1	10	15
6	1	2	3
7	1	1.47	2
8	1	1.67	2.5
9	1	1.6	3.2

Tripolar Mode

Experiment	Tail	Urethral sphincter	Bladder
1	1	0.55	0.45
2	1	0.95	0.27
3	1	0.95	0.41
4	1	0.91	0.27
5	1	0.8	0.6
6	1	0.82	0.59
7	1	0.69	0.5
8	1	0.96	0.52
9	1	0.95	0.28

Table 11 - Ratio of current thresholds for tail muscle, bladder and urethral sphincter excitation in bipolar and tripolar modes. The current thresholds for activation of the bladder and the external urethral sphincter were presented as multiples of the current thresholds for activation of the tail muscle.

Recruitment sequence of tail muscle, urethral sphincter and bladder with bipolar and tripolar stimulation.

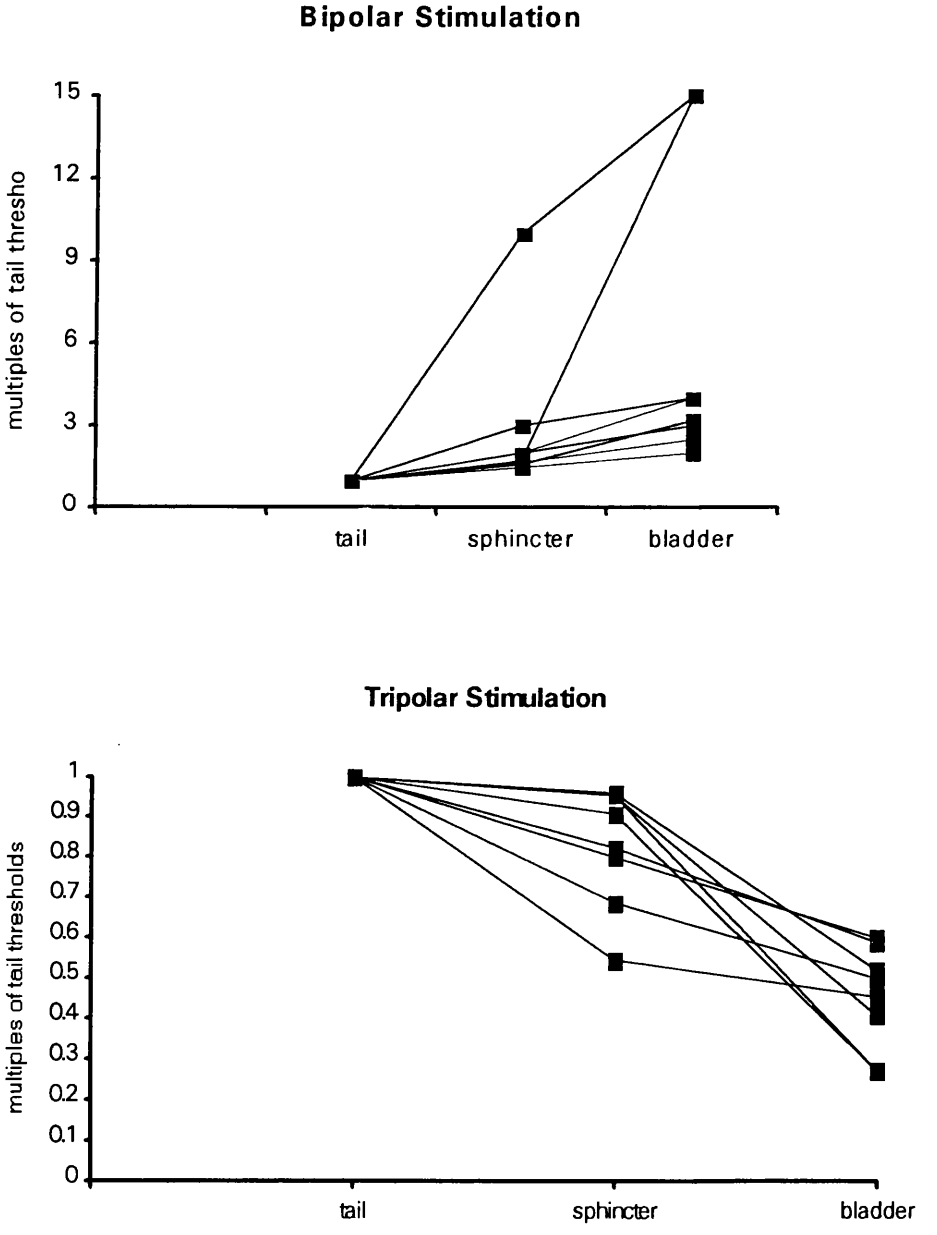


Figure 55 - Ratio of current thresholds for tail muscle, bladder and urethral sphincter excitation in bipolar and tripolar modes. The tail threshold is normalised to 1 and all the other currents are expressed in multiples of the tail threshold. The recruitment sequence obtained during bipolar stimulation is inverse during tripolar stimulation.

CHAPTER SEVEN

STATEMENT OF RESULTS

Chapter 7 - Statement of the Results

Initially, experiments were designed to investigate the blocking properties of the tripolar cuff electrode. An isolated sciatic nerve preparation was used during the *in vitro* experiments to compare the evoked compound action potentials recorded at both ends of the cuff electrode during bipolar and tripolar stimulation. Details of experimental methods were given before, in chapter 5.

Conventional bipolar stimulation resulted in the activation of axons and propagation of action potentials from both ends of the cuff. These action potentials were attributed to the larger myelinated nerve axons. However, with tripolar stimulation, this same population of fibres was still seen to propagate action potentials at the escape end of the cuff but no action potential was recorded at the blocking end. Although this indicates blocking of the larger diameter axons, there was no indication of selective blocking or activation of small fibres. Therefore, the experiment design was improved in order to discriminate between the response in different sizes of axons.

The ventral sacral roots (S_2 or S_3) of anaesthetised rabbits were stimulated unilaterally in acute experiments in order to investigate the selective excitation of the small autonomic fibres and the selective blocking of larger motor axons. These experiments were presented in chapter 6. The ventral sacral root was chosen because it contains mixed population of somatic and autonomic axons. The excitability of larger somatic motor axons was assessed indirectly by using averaged EMG to detect the first signs of skeletal muscle activity. The possibility of selective activation of the smaller skeletomotor fibres supplying the external sphincter and the autonomic motor fibres supplying the bladder was tested using pressures recorded during bipolar and tripolar ventral

sacral root stimulation. The rabbit was confirmed as a suitable experimental model. It was possible to perform the necessary urodynamic evaluations. The sacral roots show to contain axons whose diameters were compatible with the ones studied in the model.

During bipolar stimulation it was possible to repeat the conventional recruitment sequence. The larger somatic axons were activated at lower threshold than the smaller autonomic axons. The consequence in the lower urinary tract was that the external urethral sphincter was activated at lower thresholds than the bladder. However, as described in chapter 1, the function of the lower urinary tract relies on the integrated response of three different groups of nerves: the parasympathetic, the somatic and the sympathetic neurones. The storage and periodical elimination of urine depend upon the maintenance of the urinary bladder as a reservoir and the urinary flow resistance offer by the bladder neck, urethra and striated sphincter. Therefore, bipolar stimulation is not suitable for controlling the lower urinary tract. Conventional bipolar stimulation of ventral sacral root produced the expected activation of external urethral sphincter prior to bladder contractions. This leads to a troublesome dyssynergia between bladder and urethral sphincter which requires further modification of the stimulation pattern to allow voiding or another technique to block sphincter activity.

With tripolar stimulation it was possible to reverse the conventional recruitment sequence in the animal model by activating selectively the smaller autonomic axons which innervate the bladder whilst blocking the larger motor axons which innervate the external urethral sphincter and skeletal muscle. In addition, the bladder was activated with less current than during bipolar stimulation. The use of lower current intensity will reduce the possibility of nerve damage

during chronic stimulation. These conclusions are supported by statistical analysis. However, in almost all experiments it was necessary to use more current than predicted by the model.

The *in vivo* experiments were designed to show the selective activation of the small parasympathetic axons by selectively blocking the larger somatic axons during tripolar stimulation of ventral sacral roots in rabbits. During tripolar stimulation, bladder can contract without any concurrent external sphincter response, so allowing the possibility of low pressure voiding. The experimental results worked generally in line with the model predictions, although more intense currents were needed than the model predicted.

CHAPTER EIGHT

DISCUSSION

Chapter 8 - Discussion

8.1: Are the methods used appropriate?

The general advantages and disadvantages of cuff electrodes and the variations in stimulus parameters will be discussed. In addition, the appropriateness of the animal model and the specific action in the lower urinary tract of the anaesthetics used will be discussed.

8.1.1 - Electrode Design and Construction

Cuffs are one of the most commonly used types of implanted electrode (Brindley et al, 1982; Mortimer, 1990; Naples et al, 1990; Rijkhoff et al, 1994). They can be employed to excite or to block nerve axons. Cuffs consist of one or more ring electrodes embedded on the inner surface of an insulating tube and are designed to wrap around a nerve fibre. Cuff electrodes may be monopolar, bipolar or tripolar. Monopolar electrodes consist of a cathode within an insulated cuff. The anodal current will be created externally. However, this external current may cause unwanted excitation of nearby tissues. Bipolar cuff electrodes consist of two ring electrodes. Current flows within the cuff between these two electrodes. When stimulated by monopolar or bipolar electrodes, axons propagate action potentials away from the cathode in both directions. There may be external current flow outside a bipolar cuff. Tripolar electrodes consist of three rings electrodes with a cathode in the middle of two anodes. The current will flow between the cathode and the anodes. The distance between the cathode and the anodes may affect the amount of current flowing. If the two anodal currents are

equal and the two anodes are equally spaced from the cathode, an anodal block may occur at both ends of the cuff. If the current through one anode is reduced and the space between this anode and the cathode is increased, it is theoretically possible to elicit an action potential which propagates past the anode so allowing unidirectional escape (Fitzpatrick, 1994).

The tripolar electrode used in this research has three platinum ring electrodes. The cathode is the centrally located ring, which is asymmetrically placed in between two anodes. The distance between the anodes and the cathode is calculated to permit selective activation and blocking in accordance with the model predictions. The two anodal currents sum together to form the cathodal current. Platinum rings were used to avoid polarisation.

The electrical field is confined within the cuff electrode so that flow of current to the neighbouring tissues is avoided. Because they are wrapped around the nerve, cuff electrodes probably also help to avoid nerve injuries due to electrode movements. Cuff electrodes have shown long term reliability (Brindley et al, 1990). The internal diameter of a cuff electrode is important. Although from an electrical point of view it is desirable a cuff which fit tightly to avoid current flow to the neighbouring tissues, surgically, a loose cuff would cause less nerve compression and oedema. Acutely, a tightly fitting cuff may cause a reduction of blood flow to the nerve and consequently nerve damage. In the long term, fibrosis can occur and it may bind the nerve and the electrode to the neighbouring tissues. In addition, the growth of fibrous tissue into the cuff can lead to long term changes in electrical thresholds characteristics and even nerve compression injuries. All these problems may interfere with the nerve response to electrical stimulation.

The Fitzpatrick Tripolar Cuff Electrode

The cuff electrode used in this research was designed by Fitzpatrick. It has three platinum ring electrodes. The cathode is the more centrally located ring, which is asymmetrically placed in between two anodes. The distance between the anodes and the cathode is calculated to permit selective activation and blocking according with the model predictions. The total electrode is 9 mm longer, the distance between the blocking anode to the cathode is 2 mm and the distance between the escape anode and the cathode is 7mm. The two anodal currents sum together to form the cathodal current. Platinum rings were used to avoid polarisation.

The main problem with the use of this design of cuff electrode is that it is an intact cylinder. There is no way of positioning the nerve within the electrode unless the nerve is cut at one end and pulled through the cuff. Therefore, it is not suitable for use in chronic experiments. However, the electrode design was suitable for acute tests as described in chapter 5 and 6. It was the case in the observations in chapter 6, where the bladder and sphincter responses obtained should be related solely with the stimulation of the distal end of the ventral sacral root. A similar electrode has been used in dogs (Rijkhoff et al, 1994). This is slightly modified to make a split-cylinder which is subsequently closed around the root. Its successful use reinforces the confidence in the tripolar stimulation strategy. Nevertheless, the long term aim of this research is to allow this technique to be used in patients. Consequently, the construction of the cuff electrode must be improved to permit to positioning the intact nerve.

8.1.2 - Variations in Response to Stimulation Related to the Methods Used during Experiments

During the experiments described in the thesis, the current needed to excite and block axons varied between experiments. Electrode induced nerve damage might be responsible for these variations in the stimulus parameters, as discussed before. This variability may be related to several factors which may alter the nerve excitability. Surgical exposure of a peripheral nerve may affect the normal structure and function of the nerve. Mechanical injury to intraneural blood vessels and nerve axons could result in various degrees of functional damage. Stretching the nerve during dissection or during positioning it in the electrode can also cause injury, which depending upon the degree of stretch could range from changes in the venous blood flow to complete intraneural ischaemia. In the more severe cases, the perineurial sheets could be damaged (Rydevik et al, 1990). Difficult laminectomies may interfere in the results by increasing the nerve manipulation so increasing the possibility of oedema and risks of nerve injury. These are acute processes and may reduce the nerve excitability or make block easier. In order to avoid trauma to the nerve and its surrounding tissues, a prudent surgical dissection is absolutely essential. Cautious handling of the nerve during dissection and during electrode implantation is essential to ensure good results with electrical stimulation.

Currents may leak from the cuff and spread in to fluids as cerebro-spinal fluid and blood. The presence of blood clots around the electrode and the sacral root may reduce the contact between them and is another possible explanation for the variation in current intensities between experiments.

8.1.3 - Animal Model

Recent work by other groups has confirmed the suitability of the rabbit as a model for urological experimentation (Brading et al, 1986; Levin et al, 1994). These were discussed before in chapter 3. In addition, the comparative anatomy shows great similarity between mammals (Barone et al, 1973; Kaplan et al, 1979). Moreover, the bladder and the urethra on rabbits are large enough to be evaluated *in vivo* using conventional methods.

Simultaneous recording of electrical and mechanical activities from rabbit detrusor muscles showed that detrusor contraction was triggered by action potentials and the level of muscular tone was determined by the frequency of these action potentials (Creed et al, 1983). Also, the action potential mechanism was mainly related to the activation of slower voltage sensitive Ca^{++} channels (Brading et al, 1986), as happens in humans.

Levin et al (1994) reports the similarities between the human and rabbit urinary tract including a comparable distribution of autonomic receptors and comparable response to activation specific autonomic receptors.

In this research, male rabbits were preferred because they were found to be easier to catheterise than female rabbits. In female rabbits the urethra and the vagina externalise through a combined external aperture, the vestibulum. It acts as a urogenital sinus, making urethral catheterization much more difficult (Barone et al, 1973; Tuffery, 1987). Nevertheless, this problem may be specific to rabbits since similar studies have been performed in female dogs (Rijkhoff et al, 1994). However, the urethra of the dog seems to be more muscular than that of the rabbits (Creasey, personal communication). The weight of the rabbit

was also chosen to allow urethral catheterization with a standard paediatric catheter.

The root distribution in the spinal canal of the rabbit is different from humans. The rabbit has seven lumbar spinal nerves and five sacral roots. The first sacral root lies on the filum terminalis, with S_2 at the level of the cauda equina. However, during the experiments, the selection of roots for stimulation was based on initial stimulation response in each experiment rather than on the basis of anatomy alone.

Only acute experiments were performed in this project. Thus, long term problems associated with rhizotomies were not encountered

8.1.4 - Anaesthetics

Anaesthetics can affect the lower urinary tract. During the experiments, spontaneous bladder and urethral contractions were reduced during deep anaesthesia, e.g. 2 to 3% halothane. The total bladder capacity increased significantly during anaesthesia with higher halothane concentrations, as can be seen in figure 36 A and B, on page 106 and in figure 37, page 108. No specific action on the lower urinary tract is reported for diazepam and nitrous oxide (Catlin, 1983). The principal effects of hypnorm and halothane are discussed below.

Hypnorm is the commercial name for a combination of fluanisone and fentanyl citrate which is a synthetic opioid (Catlin, 1983). It may cause respiratory depression. In some of the experiments reported here the rabbit experienced respiratory distress which was corrected by mechanical respiration. In the lower urinary tract, opioids may increase the tone of detrusor and urethral external sphincter, with a tendency to retain urine. However, these effects were not observed

during the experiments probably because urodynamic evaluations were performed at least 30 minutes after drug administration.

Halothane is a potent inhalation anaesthetic. Adverse effects related to Halothane use, such as lower blood pressure are dose dependent and did not interfere with these experiments. Halothane causes relaxation of smooth muscle (Miller and Katz, 1983). Indeed, urodynamic evaluations performed during the experiments confirmed a dose dependent increase in bladder compliance. This is shown in figure 36, page 106 and figure 37 on page 108. With halothane concentrations higher than 2% almost no sign of spontaneous detrusor activity was recorded.

8.1.5 - Urodynamic Evaluation

Cystometry was used to evaluate bladder response to stimulation during the experiments. Electrical stimulation of the second sacral root in rabbits contracts the detrusor. These contractions cause a rise in the intravesical pressure, as can be seen in figures 49 to 54, on pages 140 to 147. Saline was used to fill the bladder because it is easy to measure and to control. CO_2 would have been another option, but it is not as physiological as saline and the rapid gas expansion could induce changes in bladder capacity and bladder response (Costa Monteiro and D'Ancona, 1991).

The response of the external urethral sphincter to electrical stimulation was also evaluated through urodynamic methods. Since the urethral sphincter was quite small in rabbits, sometimes it was difficult to maintain the catheter in place. Consequently, the position of the catheter in the urethral external sphincter was confirmed during

experiments in several ways. The urethral pressure profile was measured to define the sphincter position. The method described by Brown and Wickham (1969) was used. It measures the pressure at different points along the urethra by monitoring the urethral resistance to a continuous and constant flow through a catheter with lateral apertures (see figure 28 and 29, on pages 92-93, chapter 5). The intravesical pressure may be transmitted to the urethra, therefore the bladder should be relaxed during the measurement of sphincter pressure

The catheter has a ridge on it near the opening for sphincter pressure measurement. This ridge could be palpated through the skin and it was used to guide the positioning of the catheter by correlation with the anatomical sphincter site and the results of the urethral pressure profile. After confirming the sphincter position by the urethral pressure profile, a mark was made in the catheter, at the level of the urethral meatus. The catheter ridge was palpated, and another mark was made in the skin. These two marks were used as guides if the catheter moved during the experiments. Another way to test if the catheter is positioned correctly is to look for a rise in the sphincter pressure during urodynamic evaluation by exerting external pressure at the point where the sphincter was expect to be.

Several others factors may interfere in the results of urodynamic evaluation measurements and they were carefully avoided during the experiments. The rate of infusion of saline used to fill the bladder and the urethra may cause involuntary contractions of these organs. Thus, the bladder was filled at a similar velocity in all the experiments. Larger volumes which overdistend the bladder may decrease the stimulation response. To avoid this, the maximum volume was kept within the range 30-40 ml. The pressure transducers were kept fixed at the same position

in all the experiments. These avoided variations in the results due to changes in the position of pressure transducer.

8.2 - Discussion of the Results

8.2.1 - Axon Diameters

Fibre size histograms were made from sections of the tibial nerve and the sacral roots in rabbits. The measurements of axons were similar to techniques describe for cats by Boyd et Davey (1968). The results are consistent with the literature and with the range of sizes studied in the model.

The calculations in Fitzpatrick model studies are based on regular cylindrical shaped axons. However, as shown in the photomontages of the tibial nerve (figure 11 on page 54, chapter 5) and the sacral root (figure 31, on page 98, chapter 6), axons can have a very irregular shape. According to Boyd (1968), the effects of shrinkage and plane of section affect the final appearance at the photomontage, but even *in natura* the axons are not completely circular. Also the thickness of myelin sheet seems to vary between axons of the same size. It is difficult to know whether or not this is a problem of plane of section, as shown in figure 56. The lack of circularity and non-uniform distribution represents a problem to the model studies. Moreover, in life, the presence of other tissues which are not included in the model calculation, e.g. fat, blood, etc. can affect the results. Therefore, the model was useful in planning the experimental tests but is not surprising

that significant difference was found between the predicted and actual current requirements. The anatomical and physiological differences in living tissues must be taken in consideration when the model is applied and some variations in the absolute values should be expected.

Effects of plans of section in a myelinated axon

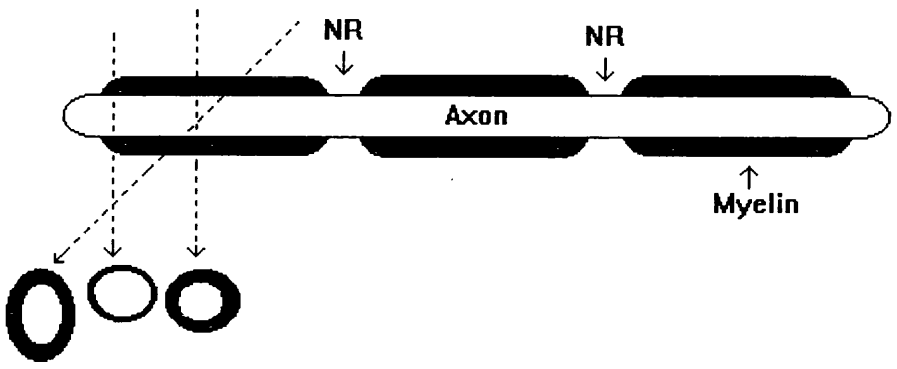


Figure 56 - Diagram of a myelinated axon showing different plans of section (broken line) and how they can interfere on the appearance of the thickness of the myelin sheet, as suggest by Boyd (1968).
NR - nodes of Ranvier

8.2.2 - Model Predictions

The results were in broad agreement with the model predictions. With tripolar stimulation a complete anodal block was achieved at the closest anode, i.e. the blocking end of the cuff, as shown in figure 16, page 63. It was also possible to activate selectively the smaller autonomic axons which innervate the bladder whilst selectively blocking the larger motor axons which innervate the external urethral sphincter and skeletal muscle at the escape end of the cuff. Block of larger motor axons at the escape anode is shown in figure 41, page 118. Selective activation of bladder during tripolar stimulation is seen in figure 51, page 143. Statistical analyses confirm that these results are reproducible.

However, in a few experiments unexpectedly high currents were needed to activate selectively the autonomic fibres. This was discussed in section 8-1. These higher than expected values may be related to several factors not anticipated in the model.

Blocking was achieved with surprisingly short pulse durations in a few cases. Table 12 shows the conduction times calculated for 2 and 7 mm for a range of axonal conduction velocities. These lengths represent the distance from the cathode to the anodal blocking end (2mm) and from the cathode to the anodal escape end (7mm). The values are calculated based in the axon's conduction velocity and the distance to be travel by them. The shaded areas represent the axons which should be blocked by 300 μs pulse. These axons are blocked because their action potentials arrived when the anodal current was still flowing, i.e. when the anodal end of the cuff is still active. The surprising experimental result is the block achieved with a 60 μs pulse duration. From the Table, fibres as fast as 30 m/s should escape past the blocking anode with this duration. These escape volleys are not seen during the experiments. But

subsequent experiments showed that the actual duration of currents flow was longer than the nominal value, as demonstrated in figure 23, page 79. The load comprising of electrode and nerve add capacitance to the stimulator and the slow decay of currents lengthens the duration of the pulse by approximately 100 μs. So, the unexpected block with shorter nominal current such as 60 μs is explained by a longer actual pulse duration.

Speed of axons m/s	Calculated times to AP arrive at the 7 mm anode or escape anode (μs)	Calculated times to AP arrive at the 2 mm anode or blocking anode (μs)
120	58	17
115	61	17
110	64	18
105	67	19
100	70	20
95	74	21
90	78	22
85	82	24
80	88	25
75	93	27
70	100	29
65	108	31
60	117	33
55	127	36
50	140	40
45	156	44
40	175	50
35	200	57
30	233	67
25	280	80
20	350	100
15	467	133
10	700	200
8	875	250
6	1167	333
4	1750	500
2	3500	1000

Table 12 - Conduction times calculated for distances of 2 and 7 mm from anode to cathode, for a range of axonal conduction velocities. The times to action potentials arrival at each anode are calculated based in the axon conduction velocity and the length they have to travel. The shaded areas represent the axons which should be blocked at 300 μs, because their action potentials arrive when the anodal current is still flowing.

The conditions of the nerve might also interfere with the blocking response. If the nerve viability is reduced, blocking may be easier and so accomplished at lower currents. On the other hand, if nerve excitability is reduced more current would be necessary to excite the nerve. In a similar way, conduction velocities may be also affected.

The ratio of anodal currents measured during the experiments did not seem to be particularly important to achieving unidirectional escape of action potentials. No clear relationship was found between the total current and the ratio of anodal current in these experiments. In practice, there was less variation in the escape current than in the total current. The control of the magnitude of the current in the escape end appears as the key point if unidirectional escape is to be achieved.

During tripolar stimulation it was possible to activate the bladder with less current than with bipolar stimulation. This is particularly important to minimise nerve damage, and others unwanted effects related with higher stimulation currents. Also, it was possible activate the bladder without concurrent urethral sphincter contraction.

8.2.3 - Problems Related to the Cuff Electrode

The difficulties of a rigid cylindrical cuff electrode for use in chronic experiments and in patients had already been discussed. However, two other problems can arise with the use of cuff electrodes: "break through" excitation and anode break excitation. They will be discussed in turn.

Break Through Excitation:

When the stimulation current applied through a cuff electrode is increased above the magnitude needed to achieve blocking, a virtual cathode may appear. This occurred in the experiment described in chapter 6. The latency of action potential in figure 42- c, on page 119, suggests the excitation occurred near the end of the cuff and not at the cathode. This event suggests the presence of a virtual cathode. Such a virtual cathode may be a consequence of potential difference created across an unbalanced cuff. The magnitude of this potential difference can be calculated using Ohm's Law, as illustrated in figure 57 and in the sample calculation below.

With a current ratio of 9:1, the potential drop across R_1 and R_2 is:

at the blocking end	at the escape end
$V_1 = 0.9i \times R_1$	$V_2 = 0.1i \times R_2$

where V is the potential in volts, i is the current in milliamperes and R is the resistance in kilohms.

Fitzpatrick (1994) reports typical values of 3.35 kΩ and 4.26 kΩ for R_1 and R_2, so:

at the blocking end	at the escape end
$V_1 = 0.9i \times 3.35\ k\Omega$	$V_2 = 0.1i \times 4.26\ k\Omega$
potential difference = $V_1 - V_2$	$= (0.9i \times 3.35\ k\Omega) - (0.1i \times 4.26\ k\Omega)$

The potential difference across the whole cuff varies with the magnitude of the current. This difference can be as larger as 12.95 V, for the maximum current possible in the experiments, 5mA, as show in Table 13-A. This potential difference sets up the external currents which cause the stimulus artefacts. If the cuff is sufficiently unbalanced a virtual cathode is created at the escape end which can excite action potentials just outside the cuff.

The potential difference decreases by changing the current ratio, such as 8:2, 7:3 and 6:4, as shown in Table 13 - B.

These results suggest that this problem could be cured either by setting the current ratio to be the inverse of the ratio of the resistance or by altering the distance between the electrodes.

Distribution of anodal currents and resistance inside the cuff electrode

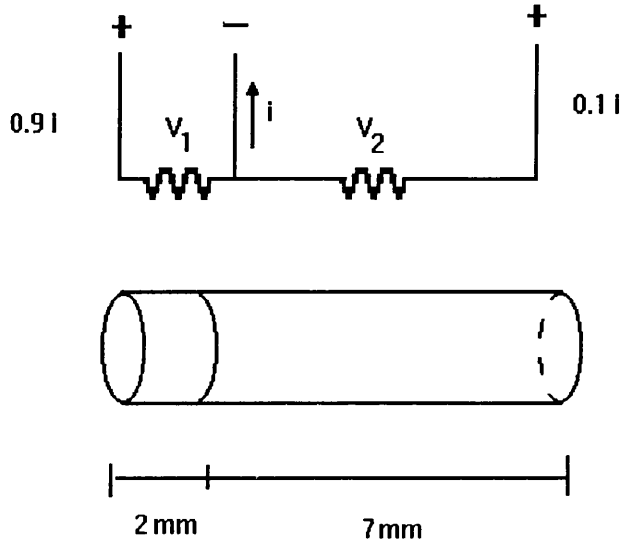


Figure 57 - Diagram represents the distribution of anodal currents and resistance inside the cuff electrode. The anodal ratio is 9:1, the distance between the blocking anode and the cathode is 2 mm, and the distance between the escape anode and the cathode is 7 mm.

A

Current	V1	V2	V1-V2
0.10	0.30	0.04	0.26
0.25	0.75	0.11	0.65
0.50	1.51	0.21	1.29
0.75	2.26	0.32	1.94
1.00	3.02	0.43	2.59
1.50	4.52	0.64	3.88
2.00	6.03	0.85	5.18
2.50	7.54	1.07	6.47
3.00	9.05	1.28	7.77
3.50	10.55	1.49	9.06
4.00	12.06	1.70	10.36
4.50	13.57	1.92	11.65
5.00	15.08	2.13	12.95

B

Ratio	V1	V2	V1-V2
9:1	3.02	0.43	2.59
8:2	2.68	0.85	1.83
7:3	2.35	1.28	1.07
6:4	2.01	1.70	0.31
5:5	1.68	2.13	-0.46

Table 13 - Potential differences across the cuff electrode.
A - Potential difference calculated based in a fixed anodal current ratio of 9:1, the total current varying from 0.1 mA to 5 mA (see formulas of potential difference).
B - Potential difference calculated based in a fixed current of 1 mA, but changing the current ratio (see figure 57). These values are calculated by changing the current ratios in the formula of potential difference.

Anodal Break Excitation

Selective excitation of small autonomic motor axons and selective blocking of larger somatic motor axons were achieved with rectangular stimulus pulses during tripolar stimulation. Rectangular pulses excite at the cathode at the beginning of the pulse and in some circumstances at the anode at the end of the pulse. Anode break excitation is more troublesome when the current intensity is high, when the rate of change of current is high and when the duration of the pulse exceeds the refractory period.

Figure 58 shows the response to stimulation with rectangular pulses in shorter and longer pulses duration. The stimulation always occurs at the cathode, at the beginning of the pulse, as represented by the stimulus artefact. If a strong current is used to stimulate, a second stimulus can rise at the anode, in the end of the pulse (S_{II}). However, after an action potential, there is a period when the axon is not excitable and no action potential can be initiated - the absolute refractory period. Therefore, for anodal excitation to occur the pulse duration should be longer than the refractory period so that the second stimulus comes at the end of the pulse with the refractory period finished.

With shorter pulse durations, no action potential can be elicited by anode break excitation (A). However, if the pulse duration is longer than the refractory period (B), anodal excitation can occur and a second action potential is fire (AP_2).

In these experiments circumstances exist in which break excitation might be expected: the current intensity can be high and when blocking occurs the axons are not refractory. However, anode break excitation was never a problem.

Stimulus behaviour in shorter and longer pulses duration

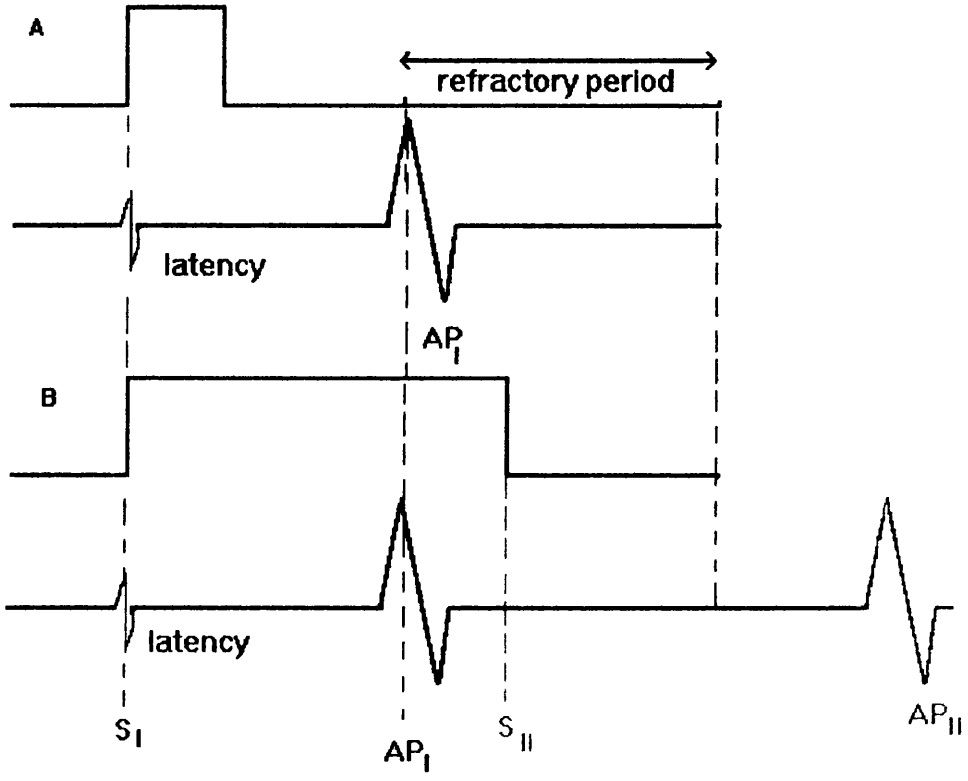


Figure 58 - Stimulus behaviour in shorter and longer pulses duration. The stimulation always occurs at the beginning of the pulse, as represented by the stimulus artefact (S_I). If a strong current is used to stimulate, a second stimulus can rise at the end of the pulse (S_{II}). This phenomena is know as anodal excitation. Anodal excitation is unlike to happen in shorter pulse duration, as shown in the first trace (A) due to the refractory period. However, if the pulse duration is longer than the refractory period (B), anodal excitation may occur.

The stimulator used during all the experiments was designed with an option to add an exponential tail to the stimulus pulse. However, because anodal break excitation was never identified, this optional capacitor was never used. Studies of the shape of pulse during experiments showed that capacitance was already accidentally added by the experimental conditions, as demonstrated in figure 23, on page 79. Although nominally rectangular pulses were used, it was demonstrated that the current pulse became rounded, with an unexpected decay at the end of the pulse. The actual duration of current flow was longer than the nominal duration. In addition, the slow current decay decreased the possibility of break excitation.

CHAPTER NINE
CLINICAL APPLICATIONS, CONCLUSIONS AND RECOMMENDATIONS TO FUTURE WORK

Chapter 9 - Clinical Application, Conclusions and Recommendations to Future Work.

9.1 - Clinical Applications

Disturbances of micturition due to neurological dysfunction is a major problem. Various solutions have been attempted and were described at the introductory chapters. Sacral root stimulation has been used as a potential new treatment. However, several factors interfere to the achievement of a completely satisfactory result. Firstly, the muscle composition of bladder and urethral external sphincter makes the lower urinary tract specially difficult to control by electrical stimulation. When the skeletal muscle in the external urethral sphincter is activated, it contracts faster than the smooth muscle. Secondly, the sacral roots contain a mixture of larger somatic axons which innervate the external sphincter and smaller autonomic fibres which innervate the bladder. The somatic fibres are activated at lower threshold than the autonomic fibres. Therefore, during bipolar stimulation of sacral roots the external urethral sphincter will contract before than the detrusor.

Attempts to evacuate the bladder during sacral root stimulation have always been difficult because of concurrent contraction of the external urethral sphincter. Detrusor contraction concurrent with sphincter contraction is a very well known problem in urology. The bladder cannot be evacuated, the intravesical pressure rises and the anti-reflux mechanism at the urethral-vesical junction will be affected. Thus, urine perhaps already infected will reflux to the ureters and kidneys. Hydronephrosis and infection follow and this can end in uraemia and death if the patient is not treated adequately.

A novel approach to bladder control problems was pioneered by Brindley and his co-workers. It uses electrical stimulation of sacral spinal roots via a bilateral array of electrodes, implanted intra or extra durally (Sauerwein et al, 1990). They attempt to achieve bladder control by judicious selection of roots for stimulation and adjustment of stimulation parameters. The Brindley sacral root stimulator (Finetech-Brindley sacral root stimulator, Finetech Ltd) is commercially available. It has been implanted in 572 patients at 42 centres in attempts to control micturition (Kerrebroeck et al, 1991 and 1993) and colorectal motility (Binnie et al, 1991). The effects of sacral root stimulation on colonic function have been studied by Binnie et al (1985-1991). The distal colon, rectum and anal sphincter are innervated by the same parasympathetic and somatic fibres which are stimulated to achieve micturition (Varma et al, 1986).

The Brindley "book" electrodes seem to be very well tolerated by tissues and have a very good success rate but like all conventional bipolar electrodes they excite large axons at lower currents than small axons. These difficulties can be partially overcome by adopting complex stimulation strategies as post-stimulus voiding. This technique is based in the physiological response of smooth and skeletal muscle. Both muscles are activate simultaneously. The external urethral sphincter, as skeletal muscle, contracts faster than the detrusor, but also relaxes much faster. By using controlled burst of pulses it was possible to sustained maximal bladder contractions with intermittent contraction of the sphincter. Therefore, there are short periods when bladder contractions overcame sphincter contractions making micturition possible during the periods of sphincter relaxation (Brindley, 1973). However, this intermittent micturition may not be able to evacuate the bladder completely. There is always a risk of urinary infection and overflow

incontinence. Moreover, bladder-sphincter dyssynergia may still be a problem in the periods when the sphincter stays contracted, increasing the risks due to high pressure in to the urinary system. One other possibility to overcome dyssynergia would be to fatigue the urethral sphincter prior to bladder stimulation (Thüroff et al, 1982). This requires higher stimulation currents for longer period which may increase the risk of nerve damage.

There is no doubt that reversing the recruitment sequence and stimulating the bladder at lower currents and without concurrent sphincter excitation is a better way to control the lower urinary tract. The results reported in this thesis demonstrate the selective activation of the detrusor by ventral sacral root stimulation in rabbits. In this research, the maximum current needed to activate the bladder with tripolar stimulation was 3mA. This produced bladder contractions up to 40 cm H_2O. These contractions have been generally strong enough to trigger micturition in the rabbits even with urethral catheterization. These results are in agreement with a similar study in the Netherlands (Rijkhoff et al, 1994). Their results also demonstrate the feasibility of selective activation of the detrusor muscle by the electrical stimulation of the appropriate sacral root. Their electrode was recently implanted in two patients (Rijkhoff, personal communication) and seem to work well during initial tests.

Rijkhoff also used monophasic rectangular pulses to achieve anodal block. Although others report the appearance of anodal break excitation with rectangular pulses (Honert & Mortimer 1981, Sweeney et al, 1989; Fang & Mortimer, 1991), this problem was only encountered once by the Dutch group. In addition, they found that the intensity of blocking was reduced by further increases in the current, probably due to a virtual cathode.

These results come together to suggest that there is a future for attempts to control the lower urinary tract by ventral sacral root stimulation. However, further developments are still needed. The cuff electrode and the stimulator will need improvements to be used more widely. Further testing in bladder response should be made regarding to its function of store urine and promote voiding.

9.2 Conclusions

1 - The experiments proved to work generally on line with the predictions of Fitzpatrick (1994). Nevertheless, the cuff electrode design is yet not suitable to be used in chronic experiments and particularly in patients.

2 - The results demonstrate that it is possible to activate selectively the bladder without concurrent contraction of the urethral external sphincter by using sacral root stimulation in the laboratory.

3 - This selectivity was achieved by anodal blocking of the somatic motor axons which innervate the urethral sphincter whilst allowing activation of the autonomic axons which innervate the bladder in rabbits.

4 - The control of the magnitude of the current flow at the escape end was probably the most important factor to achieve selectivity.

9.3 - **Recommendations to Future Work**

The present study in rabbits shows that the bladder can be activated with sacral root stimulation without the concurrent external urethral sphincter activity, at least in the laboratory. The currents needed to trigger bladder contractions were smaller with tripolar stimulation than with bipolar stimulation. The intravesical pressures achieved during tripolar stimulation seem to be strong enough to trigger micturition. All these are very encouraging results, particularly if there is a possibility of this being reproduced in patients in the future.

However, there is still a large gap between the laboratory results and the applicability in humans. There are some important questions to be answered before progress can be made to a clinical use of these techniques.

The cuff electrode needs some refinements. A balanced cuff may avoid the problems of a virtual cathode at the end of the cuff and prevent long-term problems with the anodal blocking. Also, the cuff needs be reduced in size to make implantation easier. The cuff needs to be split to allow nerve position without the need of proximal nerve division. Further tests should be made to study the urinary flow and if a complete voiding can be achieved during tripolar stimulation. Finally, bilateral stimulation of ventral sacral root may be more effective than unilateral stimulation and should be tested.

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