Sacral root afferent nerve signals for a bladder neuroprosthesis

from animal model to human

Kurstjens, Mathijs

Publication date:
2008

Document Version
Publisher's PDF, also known as Version of record

Sacral root afferent nerve signals for a bladder neuroprosthesis: From animal model to human

Ph.D. thesis

By

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2008

ISBN 978-87-7094-005-4

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Preface and acknowledgements

This thesis is based on studies performed at the Center for Sensory-Motor Interaction (SMI) at Aalborg University (Denmark). The animal experimental work of these studies was performed at the Institute for Experimental Clinical Research of Århus University Hospital Skejby (Denmark), and the clinical experimental work was performed at the Institute Guttmann, Neurorehabilitation hospital of Badalona, Barcelona (Spain).
The studies presented in this thesis were made possible by grants from the Danish National Research Foundation, the European Commission (NeuroPRO and REBEC projects) and the Generalitat de Catalunya (Catalan network "Neuroprosthesis for rehabilitation", Spain).

First of all, I would like to thank my supervisor Professor, dr. med. Thomas Sinkjær and co-supervisor dr. Nico Rijkhoff for bringing me to SMI and providing support throughout the study. Also for staying available on the background when at the end things took a bit longer then originally planned.

A large proportion of the studies described in this thesis were performed in an animal model. This work was done together with dr. Asger Dalmose who performed the implant surgeries in the pigs as well as assisted in the numerous follow-up experiments. Asger, it has been a great time working together with you, thanks a lot. Also thanks to the staff from the institute at Skejby Hospital where the pig experiments were performed, as well as the people at the farm, Påskehøjgård, for taking good care of the chronic pigs between follow-up experiments.
A thesis project with different aspects to it as this one could never have been completed without the help and advice from many other people. Persons currently or at some time in the past working at SMI who I would like to thank are dr. Ken Yoshida, dr. Morten Haugland, dr. tech. Dejan Popovic, dr. Hans Struijk, dr. Francisco Sepulveda, dr. Dario Farina, and Jan Stavnshøj. From Aalborg Hospital, Department of Pathology, I would also like to thank dr. med. Karsten Nielsen who was always willing to receive me when I had obtained new nerve specimens for histological examination.
A word of thanks also to the people at the Institute Guttmann in Barcelona (Spain) for their warm welcome to me and the great effort to making as many things possible as we could ask for. It resulted in a series of intra-operative recording experiments where, even though many of the surgical staff hardly spoke English, great results were obtained. In particular, I would like to mention the urologists dr. Borau and dr. Rodríguez who performed the surgical procedures and dr. Joan Vidal for the organization behind the scene. Last but not least thanks, of course, to the patients that gave consent to interrupt their surgery for us to perform our recording experiments.

Finally, a special word of thanks to friends and my family, especially to my wife Erika for her love, support, advice, and the many hours in evenings and weekends she had to do without me when I was working on the thesis, as well as more recently the strong encouragement from my newborn son Lucas to finally finish the thesis so that I could spent more time at home with him.

Mathijs Kurstjens
Aalborg, February 2008

Summary

Neurogenic detrusor overactivity (NDO) is a common form of bladder dysfunction in patients with neurological disorder or spinal cord injury. It causes a failure of the storage function of the lower urinary tract and is characterized by involuntary bladder contractions at relative low volumes. If left untreated, NDO can lead to low bladder capacity, incontinence, high intravesical pressure, and reflux of urine causing kidney damage. Conventional treatments are often unsuccessful and may have severe side effects.

Alternatively, NDO could be managed by electrical stimulation of appropriate afferent nerve fibers to activate existing spinal inhibitory systems that are capable of interrupting a detrusor contraction. This implies that a sensor is needed to detect the onset of a contraction. Previous studies in acute animal models have shown that afferent nerve activity associated with mechanical activity of the bladder can be recorded from cuff electrodes placed on peripheral nerves that innervate the bladder and used as such a sensor. The main objectives of this thesis were implantation the cuff electrode for recording sensory nerve signals from the bladder in a chronic animal model and to make the transition from using animal models to perform the first clinical study.

Cuff electrodes were implanted on the extradural sacral root in mini-pigs. The state of the neural interface was evaluated regularly based on evoked compound action potentials and cutaneous nerve activity and after conclusion of the implants nerve sections were examined for possible histological changes. The duration of implantation varied across animals from 19 days to more than one year. The results showed that success mainly depended on the amount of damage that was inflicted to neural structure during or after electrode implantation. The extradural sacral root was found to be very susceptible for nerve damage because of the limited space available for the cuff electrode. On the other hand, one implant was successful for more than one year, indicating that long-term implantation of a cuff electrode for recording of sacral root sensory nerve signals is feasible.

At the follow-up experiments, nerve activity was also monitored during mechanical stimulation of the sacral dermatome, bladder filling, and rectal distensions. During initial experiments, where the animals were anesthetized, nerve responses from the bladder and rectum were present but much smaller than those from the dermatome. During later experiments in the awake animals, cutaneous nerve responses were still present but responses from the rectum and specifically bladder were more difficult to obtain because of increase background nerve and muscle activity. Nerve activity recorded during conscious cystometries correlated well with the pig's voiding behavior in general but not with activity of the bladder itself, indicating that the contribution from bladder afferents to the aggregate activity during natural behavior is too small to obtain a correlation between bladder pressure and recorded whole nerve signal.

A clinical study to investigate the feasibility of recording sacral root nerve activity in human was also preformed. A cuff electrode was temporarily placed on an extradural S3 sacral root in spinal cord injured patients undergoing surgical implantation of a FineTech-Brindley bladder system. Using an experimental protocol nearly identical to the pig studies, it was demonstrated that also in human increases in whole sacral root nerve activity can be recorded in response to mechanical stimulation of the bladder, rectum and relevant dermatome.

In a pilot study supervised classification of afferent nerve activity from the bladder rectum and skin was performed using nerve signals recorded from two SCI-patients. Classification was based on a feature space obtained from a discrete wavelet transformation of the neural signal. It was found that cutaneous nerve signals could be distinguished from nerve signals from the rectum and the bladder with an error of respectively 12.8% and 24.6%, but signals from the latter two sources were more difficult to be distinguished from each other (36.6% error).

The results in this thesis showed that, when taken the necessary precaution, afferent nerve activity from different pelvic organs can be recorded using cuff electrodes chronically implanted on the extradural sacral roots. Furthermore, it was demonstrated that results similar to those obtained previously in acute animal models are also feasible in human. However, the similarities share also the same main shortcoming: lack of selectivity towards afferent nerve activity originating from the bladder. Improvements through more advanced signal processing techniques, a different location of electrode application and improved electrode design are needed before clinical application is possible.

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Chapter 1

Introduction

1 Background

Maintenance of urinary continence and voiding are daily functions that are learned and developed into social acceptable behaviors during early childhood. Bladder emptying can be voluntary postponed and initiated at an appropriate and socially convenient time. Mature toilet behavior requires the closest possible integration of the autonomic and somatic nervous systems at both the conscious and subconscious levels of neural control (Craggs and McFarlane 1999).

The process of urination is performed by the lower urinary tract (LUT) and involves two phases: the filling or storage phase, and the emptying phase or voiding. The LUT consists of the bladder and an outlet structure, formed by the urethra and urethral closure muscles, which exhibit a reciprocal relation in effecting the storage and voiding function. Damage or disease in any of the neural pathways controlling the LUT can disrupt these functions and cause urinary incontinence, most commonly as a result of detrusor overactivity (Abrams et al. 2002).

Detrusor overactivity is defined as involuntary contractions of the detrusor muscle during the filling phase which may be spontaneous or provoked and, according to cause, it may be classified as either neurogenic detrusor overactivity (NDO) when there is a relevant neurological condition or idiopathic detrusor overactivity when there is no defined cause (Abrams et al. 2002). The most common site at which damage to the nervous system causes bladder dysfunction is the spinal cord (Brady and Fowler 2001). Injuries of the spinal cord are devastating and, because of industrialization and motorized transport, unfortunately common. For example, in the Unites States approximately 10,000 new cases of spinal cord injury (SCI) occur annually adding to an already existing population of 250,000 patients with such injuries (DeVivo 1997). In Europe, the prevalence is similar with at least 330,000 people living with SCI and about 11,000 new cases occur every year (Ouzký 2002). In Denmark, an incidence of approximately 47 new SCI-patients per year has been reported for the period 1975-1984 (Biering-Sorensen et al. 1990).

NDO is a common form of bladder dysfunction in patients with SCI and occurs because of an interruption of the neural pathways between the brain and lower part of the spinal cord. It causes a failure of the storage function of the lower urinary tract. Often the synergy between bladder and sphincter function is also lost and they contract simultaneously instead (detrusor-sphincter dyssynergia). If left untreated, NDO can lead to low bladder capacity, incontinence, high intravesical pressure, and death when reflux of urine causes kidney damage and failure (Selzman and Hampel 1993). In the past, renal failure was the primary cause of death in SCI patients but advances in urological management over the past decades have significantly reduced the incidence of urinary related deaths such that nowadays they rank only fourth after respectively respiratory, heart and injury related deaths (Frankel et al. 1998).

Although most patients with neurogenic bladder dysfunction can be managed with conservative therapies, a considerable amount of patients continue to have significant urological problems even though maximal therapy is applied (van Kerrebroeck 1998). Hence alternative methods for urological management are needed to improve the quality of life of those patients. Apart from medical reasons, bowel and bladder control is also the function that individuals with SCI would most like to regain (Becker et al. 2003). The studies described in this thesis are directed towards the development of a new implantable device to manage NDO in those SCI patients in whom conservative management therapies have failed.

2 Towards an implantable neuroprosthesis for management of NDO

To which degree sensory and motor function is permanently lost after a spinal injury depends on the completeness and level of the lesion. Although the nerve fibers whose cell bodies are damaged at the level of the lesion die and atrophy occurs in the muscles that lost their innervation, the peripheral neuromuscular system below the level of the lesion is often intact. Functions that are lost or diminished because of the injury could be replaced or augmented by artificial electrical stimulation of the appropriate neuromuscular tissue that has remained intact (Mortimer 1981; Popovic and Sinkjaer 2000). Neuromuscular activation can be achieved by applying electrical pulses to motor nerve fibers innervating the muscles, directly to the muscle itself in case of deinnervated muscle, or to sensory nerve fibers to activate reflex pathways. A system using functional electrical stimulation (FES) is commonly named neuroprosthesis and, depending on the location of stimulation, it is used as an external or implantable device. Neuroprostheses have been developed for restoring function in the upper extremity, lower extremity, bladder and bowel and respiratory system (Peckham and Knutson 2005).

Electrical stimulation may also be used to suppress overactivity of the bladder during the filling phase by activating existing spinal inhibitory systems capable of interrupting a detrusor contraction. These inhibitory systems normally prevent involuntary leakage during defecation, coitus and physical activity (Lindstrom and Sudsuang 1989), but can also be activated by stimulation of appropriate afferent nerve fibers in anorectal branches of the pelvic nerve (Vodusek et al. 1986), the dorsal penile/clitoral nerve (Wheeler, Jr. et al. 1992), and the dorsal sacral nerve roots (Bosch and Groen 1998). Although several studies have shown that electrical stimulation of these afferents may have long lasting effect on bladder inhibition in non-neuropathic bladder dysfunction (Fall and Lindstrom 1991), this is not the case in neuropathic bladder dysfunction where chronic stimulation is needed (Previnaire et al. 1998). Stimulation does not need to be applied continuously but is only needed when a detrusor contraction occurs. Conditional stimulation has been shown effective in inhibiting reflex contractions evoked by rapid saline infusions (Shah et al. 1998), during cystometries (Dalmose et al. 2003; Kirkham et al. 2001), and during natural filling (Hansen et al. 2005).

For conditional stimulation to be feasible as a treatment option, a safe and reliable method for monitoring intravesical pressure on a long-term basis is necessary. Implantable sensors with sufficient long biocompatibility and reliability are difficult to build and biocompatibility, tissue erosion, sensor attachment to the bladder wall and incrustation can be a problem in implantation (Koldewijn et al. 1994; Mills et al. 2000). However, with the advent of methods for long term electrical interfacing with nerves, recording from the natural sensors in the human body have become a realistic alternative (Sinkjaer et al. 1999). These sensors are readily available and still

functioning in patients with a spinal injury, only the transmission of afferent signals is interrupted at the lesion. Combining the available natural sensory and inhibitory systems innervating the urinary bladder with neural recording, signal processing and electrical stimulation techniques, a closed-loop neuroprosthetic device to control the overactive bladder has been proposed (Jezernik 2000). Such a system would be able to detect the onset of involuntary bladder contractions based on sensory information recorded from a nerve that innervates the bladder (see Figure 1). Detected bladder contractions are subsequently suppressed by applying electrical stimulation to activate appropriate inhibitory neural pathways.

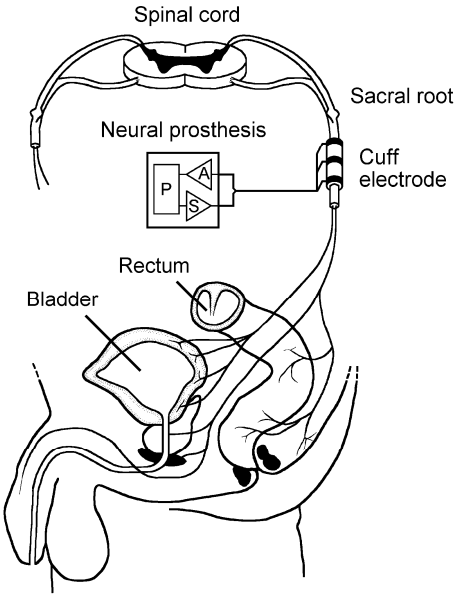

Fig. 1: Neuroprosthetic device for treatment of NDO interfacing the nervous system through a cuff electrode placed on a sacral nerve root innervating the urinary bladder. The device principally contains an amplifier for nerve signals (A), a signal processing unit (P), and an electrical stimulator (S).

If sensory information from the bladder is available, it may also be possible to determine the bladder volume. In SCI-patents, bladder emptying is usually performed in a time-dependent manner. Physiological changes in urine production as a result of, for example, fluid intake or temperature alterations (Klevmark 1999) may therefore lead to early emptying attempts or to bladder overdistention when the bladder capacity has been increased by inhibition of undesired detrusor contractions. Instead, bladder emptying could possibly be more volume-dependent and on self-indication as bladder-filling sensation is still present to some degree in many complete SCI-patients (Ersoz and Akyuz 2004), but emptying would often not immediately be possible and some warning time may be appreciated.

Therefore, by combining neurostimulation techniques already in clinical use for bladder emptying (Brindley 1994) with natural sensory feedback in one device, an advanced implantable neuroprosthesis can be created that is able to restore the lower urinary tract function to a state similar to normal.

3 Aims and overview of the thesis

Recent studies have demonstrated that the activation of natural sensors in the bladder, produced by increases in bladder pressure during bladder contractions and passive bladder distensions, is reflected in the whole nerve activity that can be recorded using cuff electrodes placed on the pelvic nerves and sacral nerve roots in acute animal models (Jezernik et al. 2000; Jezernik et al. 2001). The research described in this thesis aims to extend these previous acute animal studies to a chronic animal model to investigate whether the same sensory nerve signals can be recorded on a long-term basis. Furthermore, it also aims to make the transition from animal models to the clinic by performing recordings in patients with spinal cord injury.

The following research questions will be addressed in this thesis:

1. Is it possible to implant cuff electrodes on the sacral roots for the purpose of recording of sensory nerve activity in a chronic animal model?
2. What kind of signals can be recorded using a cuff electrode implanted on a sacral nerve root in a chronic animal model and how do they relate to specific physiological events, in particular mechanical activity of the bladder?
3. Is it possible to record afferent nerve activity related to mechanical bladder activity by means of cuff electrodes placed on sacral nerve roots in human?
4. Is it possible to identify the recorded human afferent nerve activity accordingly to its origin?

A large portion of the work described in this thesis concerns the implementation of a cuff electrode in a chronic animal model. Because there was no previous experience with chronic recording from the sacral nerve roots, the chronic animal work was divided into two separate studies: the effect of the chronic cuff implantation on the neural interface (research question 1) was investigated separately from a study into the different signals that were recorded from the sacral roots during the implantation periods (research question 2). In chapter 2, implantation of cuff electrodes on the extradural sacral roots in mini pigs together with a telemetric device is described. This allowed the recording of nerve activity on follow-up experiments with the animal either anesthetized or conscious. The stability of the neural interface was investigated using artificial and natural stimulation of sacral root sensory nerve fibers and the histology of the nerve tissue enclosed by the cuff was evaluated with regards to possible nerve damage after the conclusion of the implantation period.

In chapter 3, the relation between the signals recorded from the implanted cuff electrodes and different events (research question 2) is investigated. Nerve activity was initially recorded in the anesthetized pig during stimulation of mechanoreceptors in the bladder, rectum and relevant dermatome but in the awake pig nerve recordings were later also performed during cystometries and reflex rectal activity.

Results obtained in the animal studies need to be confirmed in human experiments if recording from the natural sensors is to be used in a device to treat patients. Chapter 4 presents therefore the first clinical study investigating the possibility of recording sensory nerve activity related to mechanical activity of the bladder from the sacral roots in human (research question 3). Nerve activity was recorded intraoperative using cuff electrodes temporarily placed on the extradural S3 sacral root in SCI-patients who were operated for the implantation of a device for bladder control.

The complexity of the nerve activity recorded in the unanesthetized pigs compared to the nerve activity that was recorded in the anesthetized pigs suggested that more sophisticated signal processing methods would be needed to be able to identify the correct sensory origin of the recorded activity. For that reason, a method of signal classification based on signal-dependent discrete wavelets was tested in chapter 5 using nerve signals obtained from SCI-patients in the preceding chapter.

Finally, a summary with discussion and conclusions, comments on methodological considerations and suggestions for future directions are presented in chapter 6.

The remainder of this chapter provides an overview of the anatomy, physiology and neural innervation of the lower urinary tract, neurogenic detrusor activity and previous work related to the recording of afferent nerve activity from the bladder.

4 The lower urinary tract

4.1 Anatomy and physiology

The function of the lower urinary tract (LUT) is storage and periodic elimination of urine. There are two functional units that form the lower urinary tract: a reservoir, the urinary bladder, and an outlet, consisting of the bladder neck, urethra with its sphincters, and pelvic floor muscles (De Groat 1993), see figure 2 below.

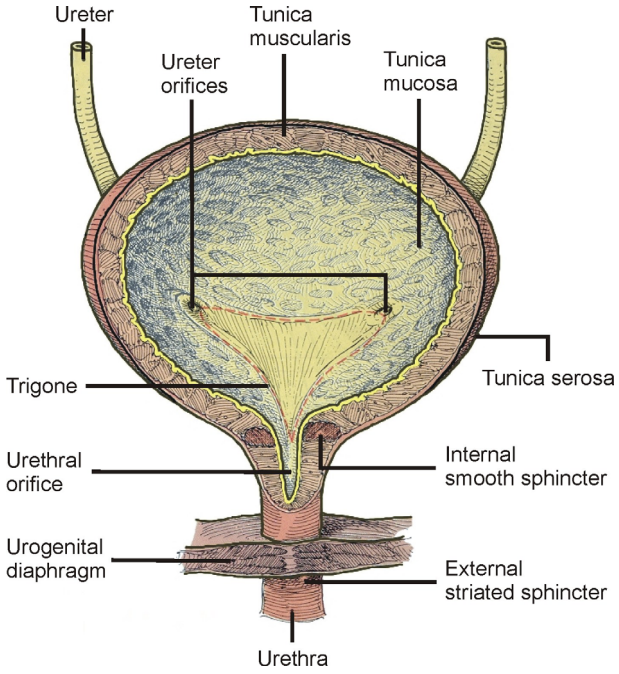

Fig. 2: The lower urinary tract. Adapted from Carola et al. (1992).

The urinary bladder is a hollow, muscular organ that via the ureters receives urine from the kidneys. It is located on the bottom of the pelvic cavity and can accumulate volumes of 300 to 400 ml with little (<15 cmH_2O) or no change in intravesical pressure (Torrens 1987). The wall of the urinary bladder is composed of tree layers (Carola et al. 1992). On the inside, it is lined with a layer of transitional epithelium (*tunica mucosa*), also known as urothelium, that forms a barrier to separate urine from the extracellular fluid and is folded allowing the bladder to stretch or

contract. The thick middle layer consists of a layered mesh of longitudinal and circular smooth muscle fibers (*tunica muscularis*), also known as the detrusor muscle. On the outside, the *tunica serosa* covers the upper and lateral surfaces of the bladder. The *trigone* is the area outlined by the openings of the ureters and urethra into the bladder cavity (bladder neck/base). A thickening of the bladder smooth muscle at the proximal urethra forms an internal sphincter, which is supported by an external sphincter consisting of the striated musculature of the pelvic floor, to maintain continence.

Under normal conditions the urinary bladder and outlet exhibit a synergistic function. During storage, the urethral sphincters are active to keep the outlet closed and the detrusor muscle is relaxed, allowing the bladder to expand slowly and maintain a low intravesical pressure. At the onset of micturition (voiding) this functional state is inverted by first relaxing the internal and external sphincters, immediately followed by contracting the detrusor muscle, raising the intravesical pressure. When the intravesical pressure exceeds the urethral pressure, the urine is propelled outward through the urethra.

4.2 Neural innervation

The central nervous system innervates the LUT through three sets of peripheral nerves: pelvic nerves, hypogastric nerves and sympathetic trunk, and pudendal nerves (De Groat 1993). Sympathetic preganglionic fibers emerging from thoracolumbar spinal segments (L11-T2) pass to the sympathetic chain ganglia and then to ganglia in the hypogastric and pelvic plexi (fig 3). Postganglionic fibers provide an excitatory input to smooth muscle of the urethra and bladder base, an inhibitory input to the bladder body, and both inhibitory as well as excitatory input to vesical parasympathetic ganglia. Parasympathetic preganglionic fibers emerging from sacral spinal segments (S2-S4) pass in the pelvic nerves to ganglia in the pelvic plexus and the bladder wall. These ganglia provide excitatory input to the bladder smooth muscle. The external urethral sphincter receives efferent innervation from somatic fibers in the pudendal nerve. Branches of the pelvic and pudendal nerves also innervate other muscles in the pelvic floor.

The majority of sensory information from the bladder body and the rectum is carried by the parasympathetic pelvic nerve afferents to sacral spinal cord segments (S2-S4), but some sensory information from the bladder base and urethra is also conveyed in the sympathetic hypogastric nerves to thoracolumbar spinal segments (T11-L2) (Janig and Morrison 1986). Sensory innervation of the distal urethra, anal canal, and perineum originates in the sacral spinal segments (S2-S4) but travels in the somatic pudendal nerve.

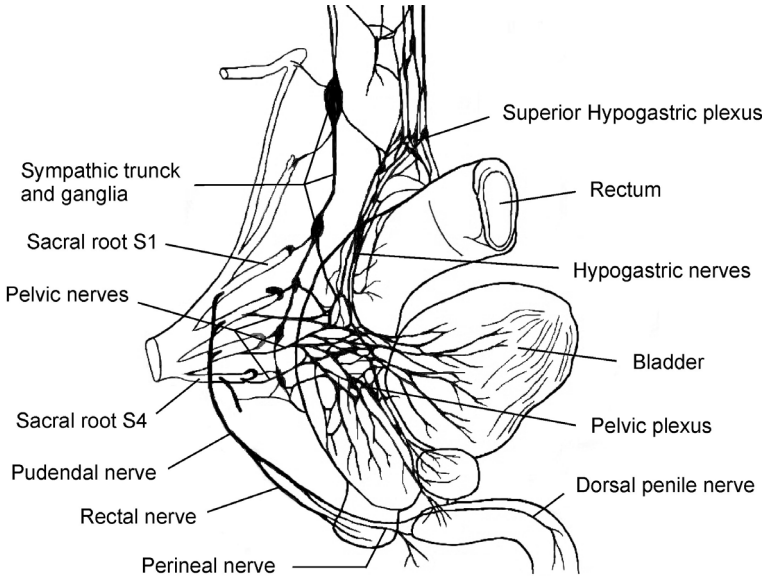

Fig. 3: Peripheral neural innervation of the male lower urinary tract. Based on Netter (1996).

4.3 Control mechanisms

The central pathways controlling the LUT function have been postulated to be organized as simple switch circuits, maintaining a reciprocal relationship between the urinary bladder and urethral outlet (De Groat and Booth 1984; De Groat and Kawatani 1985). Proper function is mainly controlled through reflex pathways that involve neural circuits, located within different levels of the spinal cord and the brain.

During the initial part of normal bladder filling, the intravesical pressure is low (< 10 cm H_2O) because of the bladder wall compliance (Craggs and Vaizey 1999). Further filling distends the bladder wall and evokes a low level of pelvic nerve afferent activity that initiates a spinal-lumbar reflex pathway stimulating sympathetic outflow to the bladder outlet (base and urethra) and pudendal outflow to the external urethral sphincter (the 'guardian reflex', fig 4A), which is supported by sympathetic inhibitory outflow to the detrusor muscle and bladder wall ganglia. An area in the ventral pons called the pontine storage center also produces a continuous excitatory output to the external urethral sphincter to keep the outlet closed (Blok 2002).

Afferent activity, first from the bladder wall and at higher bladder pressures also from the bladder neck (posterior urethra), evokes the micturition reflex (Craggs and Vaizey 1999; Guyton 1991). The pattern of efferent outflow is then reversed by excitation of parasympathetic pathways to the bladder, and inhibition of the somatic pathways to the urethra sphincter (De Groat and Kawatani 1985). Micturition starts with an initial relaxation of the urethral sphincter, immediately followed by contraction of the bladder smooth muscle. Secondary reflexes elicited by afferents that are sensitive to flow in the urethra facilitate bladder emptying (Kuru 1965). The reflexes mediating excitatory output to the sphincters and sympathetic inhibitory outflow to the bladder are organized in sacral and thoracolumbar segments of the spinal cord, whereas the organization of the parasympathetic outflow to the detrusor has a more complicated organization involving different areas in the brain stem (pontine micturition center), cerebral cortex, and hypothalamus (De Groat 1993).

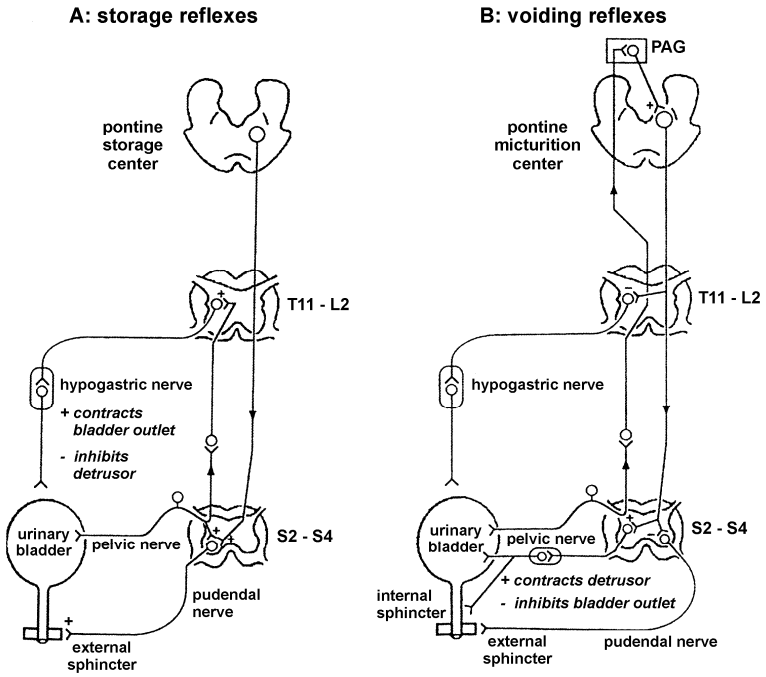

Fig. 4: Diagram illustrating the different neural pathways of the reflex mechanisms controlling the (A) storage and (B) voiding phase of the micturition cycle. See text for detailed description. Adapted from De Groat et al. (1999).

The cerebral involvement in the micturition reflex (spino-bulbo-spinal reflex) includes the projection of bladder afferent pathways to the periaqueductal gray substance (PAG in fig. 3B), which in turn projects to the pontine centers of the brain stem (Blok et al. 1995). From these centers originate descending excitatory and inhibitory pathways that modulate the lumbosacral segmental reflexes coordinating bladder and sphincter function (De Groat 1993; Morrison 1987)

5 Neurogenic detrusor overactivity

5.1 Causes of neurogenic detrusor overactivity

Normal physiological bladder control requires the involvement of different central and peripheral neural circuits. Injury or disease of the nervous system, as well as drugs or disorders of the peripheral organs, can therefore cause bladder dysfunction. Common neurological disorders associated with the LUT dysfunction include multiple sclerosis, cerebral vascular accidents, Parkinson's disease, spinal cord injury (SCI), herniated discs, and diabetes mellitus (Chancellor and Blaivas 1995a). These disorders affect bladder function in different ways depending on the level and site of the neurological impairment.

Neurogenic detrusor overactivity has been associated with neurological lesions at a suprapontine, suprasacral, sacral, and peripheral level of the nervous system (Blaivas and Chancellor 1995). A lesion at suprapontine level caused by, for example, a cerebral vascular accident, Parkinson's disease, brain tumor or trauma, removes the inhibitory control of the higher brain centers on the pontine micturition center. This results in loss of voluntary control over the micturition reflex, leading to uninhibited detrusor contractions but with detrusor-sphincter synergy. However, the bladder can be contracted voluntary and bladder sensation is also intact. Neurological disorders, such as SCI, transverse myelitis or multiple sclerosis,

interrupt the spinal pathways between the brain stem and the sacral micturition center, resulting in uncoordinated micturition. Involuntary detrusor contractions occur, often with detrusor-sphincter dyssynergia, and sensation from the bladder depends on the completeness of the lesion. Lesions at or below the level of the sacral cord segments because of, for example, cauda equina injury, pelvic surgery/trauma, or diabetes mellitus, result in an areflexive bladder with incomplete emptying and large residual volume.

In case of a suprasacral spinal cord injury, the initial response is a sudden cessation of spinal reflex activity in areas below the level of injury (spinal shock), leading to an areflexic bladder (De Groat 1995). In contrast, the sphincter activity persists or recovers rapidly causing urinary retention. During recovery from spinal shock, usually within a few weeks after injury, involuntary detrusor contractions appear in response to visceral stimuli, such as bladder filling or pressure increases exerted externally to the bladder (Yoshimura 1999). Initially, these detrusor contractions are not sustained and generate low intravesical pressure, but over time they become more powerful and can produce involuntary voidings. However, voidings are usually inefficient as the bladder can only be emptied partially because of detrusor-sphincter dyssynergia. Involuntary detrusor contractions with detrusor-sphincter dyssynergia result in large residual volume and high intravesical pressure, leading to frequent urinary infections and renal damage due to reflux nephropathy (Arnold 1999; Chancellor and Blaivas 1995b). A noxious visceral stimulus from below the level of injury, such as high-pressure involuntary detrusor contractions, bladder distension or urethral catheterization, can also trigger an episode of autonomic dysreflexia. Symptoms include sweating, flushing above the level of injury, reflex bradycardia, and hypertension, which may cause headache and a risk for cerebrovascular accident (Arnold 1999; Chancellor and Blaivas 1995b).

The involuntary detrusor contractions are induced by a spinal reflex pathway mediated by C-fiber afferents, which may be the result of multiple mechanisms: (1) elimination of bulbospinal inhibitory pathways; (2) strengthening existing synapses or formation of new connections by axonal sprouting of C-fiber afferents in the spinal cord; (3) afferent neuron hypertrophy, expansion of the afferent terminals in the cord and facilitation of spinal reflexes, indirectly related to detrusor-sphincter dyssynergia and outlet obstruction; (4) increased sensitivity to bladder distension of high threshold C-fiber afferents because of alterations in excitability at afferent receptors in the bladder. (De Groat 1995)

5.2 Management of neurogenic detrusor overactivity

The objectives for management of NDO are to achieve continence and prevent upper urinary tract damage. This can be accomplished by restoring low intravesical pressure and large storage capacity, and ensure satisfactory bladder emptying (Arnold 1999; Craggs and Vaizey 1999). The methods used to accomplish this depend on the severeness and time since injury.

Pharmacological therapy is the main management method of NDO and different classes of agents are used depending on the target of the intervention. Targeted are mostly receptors or ion-channels known to be involved in the control of detrusor contractions, or neurotransmitters involved in the micturition reflex pathways (Andersson 1999).

Antimuscarinic drugs are currently the main drugs used to treat detrusor overactivity (Andersson et al. 2001; de Ridder and Baert 2000). They contain anticholinergic agents that suppress detrusor contractions by blocking muscarinic receptors at the neuromuscular junction. Other often used drugs are alpha-adrenergic blocking agents which decrease bladder outlet resistance and improve bladder emptying. When detrusor overactivity is more profound, pharmacological

therapy is often combined with indwelling or intermittent catheterization. In case of SCI-patients, the bladder is initially left on continuous drainage (suprapubic or urethral indwelling catheterization) during the spinal shock phase, but most patients are converted to intermittent catheterization after reflex activity appears (Arnold 1999; Chancellor and Blaivas 1995b).

When pharmacological therapy combined with intermittent catheterization is unsuccessful in reducing intravesical pressure and achieving continence, alternative and more invasive methods such as bladder autoaugmentation or augmentation cystoplasty (Chapple and Bryan 1998), urinary diversion, or deafferentation (Hohenfellner et al. 2001) can be employed. Although bladder deafferentation in the form of dorsal sacral rhizotomy of the S2-S4/5 sacral segments can be performed as an autonomous management option in selected patients (Hohenfellner et al. 2001), it is mostly performed in combination with a ventral root stimulator for emptying of the bladder in SCI-patients (see section 5.4).

Recently, intravesical administration of new drugs has become another option before going to surgery as last resort. Detrusor overactivity has traditionally been treated with drugs that act mainly on the efferent neurotransmission or the detrusor muscle itself. However, due to the increasing knowledge on the afferent mechanisms involved in the development of detrusor overactivity, transmitters of afferent nerves and their receptors have become a new target for pharmacological interventions (Andersson 1999; Yoshimura and Chancellor 2002). Intravesical instillation of capsaicin (Arnold 1999; Fowler et al. 1992) or resiniferatoxin (Cruz et al. 1997; Kuo 2003) causes a long-lasting desensitisation of mainly C-fiber bladder afferents that are thought to become predominant in evoking involuntary detrusor contractions after SCI (De Groat 1995). Another recent development is the direct injection of small doses of botulinum-A toxin into the detrusor muscle where it selectively blocks the release of acetylcholine at the intramuscular nerve terminals and thus inducing detrusor paresis (Schurch et al. 2000).

5.3 Drawbacks of current management methods of NDO

The different management methods currently available have however been advocated with variable success rates or have side effects. Success of pharmacological therapy is limited and drugs meant for treatment of detrusor overactivity often lack selectivity for the bladder (Eglen et al. 1996). Their targets may also be present in other tissues throughout the body, causing side effects such as dry mouth, constipation, blurred vision and drowsiness (Andersson 1999; Yoshimura and Chancellor 2002). During intravesical installation of capsaicin, patients with sensation suffer severe suprapubic burning sensation and pain unless injected with lidocaine before instillation (de Ridder and Baert 2000). With both capsaicin and botulinum-A toxin the effect is only temporal and the instillation or injections have to be repeated every 6-9 months. Since botulinum-A toxin paralyses the detrusor muscle, intermittent catheterization is also still needed to provide means of bladder emptying, while also severe generalized muscle weakness has been reported in a few cases. In case of bladder augmentation, the patients are still left on catheterization; the long-term use of an indwelling catheter pose a major risk for the development of urolithiasis, urinary tract infections, low bladder compliance, and even bladder cancer (West et al. 1999). Aside from the procedure specific drawbacks, each surgical procedure is of course associated with risks and disadvantages common for any surgery in general, such as for example pain, infection, and brain damage due to problems with ventilation or anesthesia.

5.4 Neuroprosthesis for management of bladder dysfunction

Methods based on electrical stimulation provide an alternative when other methods for treatment of bladder dysfunction fail, are unsatisfactory or the side effects cannot be tolerated (van Kerrebroeck 1998). Several systems using electrical stimulation are currently clinically available, each with different modalities of operation. The oldest of them was initially developed to permit evacuation of the bladder in SCI-patients and includes implantation of electrodes on the anterior sacral nerve roots (Brindley 1977). The principle of operation of this system, known as the FineTech-Brindley Bladder System, lies in the use of intermittent bursts of stimuli and difference in relaxation time of the external urethral sphincter and detrusor muscle resulting in post stimulus voiding. Although the bladder can be emptied effectively on demand and with low residual volume, continence is only restored when implantation is performed in combination with a posterior sacral rhizotomy to abolish involuntary bladder contractions (Brindley et al. 1986). The posterior rhizotomy improves bladder capacity and decreases detrusor-sphincter dyssynergia and autonomic dysreflexia, but at the same time it is accompanied by a loss of reflex erection and ejaculation in men or lubrication in women, alteration in ano-rectal reflex activity, and loss of sacral sensation in incomplete SCI (Creasey and van Kerrebroeck 1996). Benefits of this system include urination on demand, elimination of catheters, improved continence and bowel function, fewer urinary tract infections, improved quality of life and social ease (Creasey et al. 2001; Vastenholt et al. 2003) as well as significant reductions in costs of bladder and bowel management on the long-term (Creasey and Dahlberg 2001; Wielink et al. 1997).

The Medtronic InterStim® therapy is used for treatment of urinary urge incontinence, urinary retention, and significant symptoms of urgency-frequency in patients who have failed or could not tolerate more conservative treatments (Chartier-Kastler et al. 2000; Medtronic 1999; Siegel et al. 2000) Therapy is accomplished by chronic electrical stimulation of the sacral nerve to modulate the neural pathways of micturition (Tanagho and Schmidt 1988; Thuroff et al. 1983). Following successful sub-chronic test stimulation (peripheral nerve evaluation, PNE, test), patients are implanted with the InterStim system and a lead is inserted through in the previously tested sacral foramen and implanted adjacent to the appropriate sacral nerve. The mechanism of action by sacral nerve stimulation is believed to produce a modulating effect on the sacral nerve reflexes that control the detrusor, sphincter and pelvic floor muscles (Bosch and Groen 1995; Siegel et al. 2000).

Aside from the common risks involved in any surgical intervention, implantation of a neuroprosthesis device increases the risk for infection, rejection of implanted materials and nerve damage due to surgical handling during electrode placement or electrical stimulation. The incidence rate of these complications for the FineTech-Brindley Bladder System and Medtronic InterStim implantations has been low, except for surgical revisions to remedy faulty implants or incomplete deafferentation (Brindley system, (Brindley 1994), to relocate the stimulator because of pain at the subcutaneous pocket, or because of suspected lead migration (InterStim system, (Siegel et al. 2000). Other complications reported following implantation of the Brindley system are leakage of cerebrospinal fluid (intrathecal electrode placement) and loss of reflex function (although penile erection may be partially regained using the simulator) (van Kerrebroeck et al. 1996). Further adverse events observed in relation to implantation of the InterStim include transient electric shock, and change in voiding or bowel function (Siegel et al. 2000).

Chronic stimulation of the pudendal nerve has also been tested in a small group of patients with idiopathic detrusor overactivity incontinence using a BION™ miniature stimulator (Groen et al. 2005; Seif et al. 2005). A BION (Advanced Bionics Corporation, USA) is a self-contained,

battery powered and telemetrically controlled miniature stimulator that was originally intended to provide functional electrical stimulation of paralyzed muscles (Cameron et al. 1997; Loeb et al. 2001). For management of detrusor overactivity, the device can be implanted adjacent to the pudendal nerve with the help of a special tool that is inserted percutaneously trough the perineum during a minimal invasive procedure. Inspired by the BION, a similar looking model micro-stimulator (M-Micro) was constructed, but with connecting wires tethered to a external stimulator (Walter et al. 2005b). Following chronic implantation in the bladder wall and near the bladder neck (close to the pelvic plexus) in a spinal cord injured animal model, controlled bladder contractions and, in some cases, voiding was obtained (Walter et al. 2005a). The only recently developed event-driven system for treatment of urinary incontinence is the Miniaturo™. This system consists of a subcutaneously implanted battery powered unit containing a electrical stimulator and signal processor (Nissenkorn et al. 2004). The stimulator is triggered by a sudden increase in abdominal pressure and is used to activate the pelvic floor muscles as well as the sphincter detrusor reflex that inhibits bladder contractions.

5.5 Alternative methods to detect bladder contractions

As mentioned in section 2, currently available techniques for direct measurement of the mechanical activity of the bladder (pressure, force) are not suitable for long-term application. Several alternative methods to obtain information on increases in bladder pressure or the onset of a bladder contraction have therefore been investigated previously.

First of all, because the bladder is a muscular organ, detrusor electromyographic (EMG) signals reflecting its mechanical activity could possibly be recorded. However, although numerous studies have attempted to record bladder EMG, obtaining a reliable recording from the bladder is still rather elusive because the signal is dominated by activity from nearby skeletal musculature and large electromechanical artifacts generated at the tissue-electrode interface as the organ contracts or the tissue moves passively (Ballaro et al. 2003). Bladder EMG is hardly distinguishable from these artifacts because the net extracellular electrical activity in bladder smooth muscle is much smaller as contractions result from a different mechanism of cell depolarization and the random arrangements and asynchronous activation by *en pasant* neuronal connections rather than within discrete motor units as in striated skeletal muscle, limits to which degree any extracellular currents are summated (Ballaro et al. 2003; Brading 1987). The artifact problems may be solved through an improved electrode design, but previous studies involved mainly animal models and it remains unknown whether detrusor EMG may be recorded, if it exists at all, in human (Ballaro et al. 2001; Ballaro et al. 2003).

Alternatively, EMG activity can easier be recorded from striated muscles in the pelvic floor whose activity is correlated with bladder activity. Dyssynergia between bladder and urethral sphincter function in patient with SCI or neurological disorders leads to an increase in activity of the sphincter that occurs simultaneous or immediately after the onset of a detrusor contraction (Blaivas et al. 1981). EMG recorded from the external urethral sphincter muscle (Hansen et al. 2007), but also from the external anal sphincter (Wenzel et al. 2006) can therefore be used to estimate the onset of a detrusor contraction, as shown in table 1.

Source	*Algorithm*	*dPblad [cm H_2O]*	*Delay [s]*	*Species*	*Reference*
EUS EMG	RMS	2.2	0.4	Human	Hansen et al. (2007)
	KSF	3.2	0.9		
EAS EMG	CUSUM	15.7	2.8	Cat	Wenzel et al. (2006)
		3.7	-0.8	Human	
PDN ENG	CUSUM	7.3	1.4	Cat	Wenzel et al. (2005)
PVN ENG	RF-MA	2.5	--	Pig	Jezernik and Sinkjær (1998)
	CUSUM	6.4	--		
SR ENG	CUSUM	9	6	Cat	Jezernik et al. (2001)

Table 1. Alternative methods used for detection of the onset on bladder contractions based on electrophysiological measurements. EUS: external urethral sphincter, EAS: external anal sphincter, PDN/PVN/SR ENG: pudendal nerve/pelvic nerve/sacral root electroneurogram, RMS: root mean square of signal plus fixed threshold, KSF: kurtosis-based scaling function, RF-MA: moving average of rectified and low-pass filtered signal plus fixed threshold, CUSUM: weighted cumulative sum. Jezernik and Sinkjær (1998) did not report absolute detection delay times, only that the delay for RF-MA was 1.3 s shorter than for the CUSUM algorithm. Although the detection time was slightly longer for the KSF and CUSUM algorithms, they generated considerably fewer false-positives.

Another approach to detect bladder contractions investigated previously is based on the electrical activity that can be recorded from nerves that innervate the lower urinary tract, see table 1. Whole nerve activity recorded from the pelvic nerves and sacral roots reflects bladder activity (Jezernik et al. 2000; Jezernik et al. 2001), whereas whole nerve activity recorded from the pudendal nerve reflecting the dyssynergic activity of the sphincters can be used to detect bladder contractions indirectly (Wenzel et al. 2005).

Finally, some patients with NDO resulting from a neurological disease or incomplete SCI can feel the sensation of a bladder contraction. Self-controlled electrical penile nerve stimulation has been demonstrated to be feasible in one incomplete SCI-patient (Lee and Creasey 2002) and in one patient with multiple sclerosis (Fjorback 2006).

Although the latter mentioned studies demonstrated feasibility of the detection method in an acute setting, they are less suitable for chronic application because of the used methods of electrode application or lack of selectivity. Sphincter EMG can only be recorded reliably using wire electrodes installed percutaneously or using a catheter or probe installed in the urethra respectively the anus, which are no methods for long-term use because of risk of infection, pull-out of wires, user discomfort or tissue damage. Furthermore, the use of sphincter EMG is limited to patients that have both NDO and DSD, and reflex activity evoked by stimulation of the perianal region or lower limb flexor muscles may cause too many false-positive detections (Hansen et al. 2007).

The main drawbacks of the nerve signal detection methods are the invasive surgery needed for electrode implantation and the lack of selectivity towards the source or the recorded neural signals. The CUSUM algorithm was developed to detect small increase in a noisy signal (Basseville and Nikiforov 1993). The studies from table 1 with the neural approach used the CUSUM algorithm to detect small increases in whole nerve activity. However, the sacral roots

innervate not only the bladder but also the urethra, rectum, skin and many muscles in the perineum and the lower limbs. Also the pudendal nerve, formed from branch of the sacral roots, innervates multiple muscles and skin in pelvic region. This implies that the activity recorded from these nerves may contain contributions from fibers originating from many different sources. The onset of a bladder contraction therefore can only be detected from this compound signal if the contribution from respectively the bladder or urethra is sufficiently large or has such unique signal properties that it can be distinguished from other signal sources. However, in reality the recorded nerve activity at a given time instant will never originate from one single organ only.

6 Recording sensory nerve signals from the bladder

6.1 Natural bladder sensors

Sensory receptors in the bladder have originally been described as tension receptors 'in-series' or 'in parallel' with the detrusor muscle fibers (Iggo 1955). The 'in series' receptor were generally considered to respond to passive distention and active visceral muscle contraction whereas the 'in parallel' receptor only respond to passive distention (Sengupta and Gebhart 1995). The 'in series' tension receptors are actually located within the perivascular connective tissue around the muscle fascicles rather than within muscle fascicles. Because of its intrafascicular attachments, the perivascular connective tissue assumes the tension developed by the fascicles and thus behaves as to be 'in series' with the muscle fascicles (Fletcher and Bradley 1970). Indeed, a suburothelial layer of spindle-shaped cells resembling myofibroblast has been found recently in the human bladder that differed distinctively from flat epithelial cells and detrusor cells and it was suggested that these myofibroblast may be involved in the transfer of information between the urothelium and suburothelial nerves (Sui et al. 2004).

Also the urothelium itself possibly plays an active role in sensory functions (De Groat 2004). Urothelial cells express various receptors and ion channels, and are able to release neurotransmitters in response to stimuli. Under mechanical force, exerted by stretch during bladder filling, substances may be released from urothelial cells that act on submucosal afferent nerve fibers.

6.2 Bladder afferent nerve fibers and their properties

Information from the sensory receptors in the lower urinary tract is conveyed to the spinal cord by afferent nerve fibers that have their cell bodies located in dorsal root ganglia. Afferent nerve fibers supplying the bladder have been identified suburothelially as well as in the detrusor muscle (Andersson 2002). Immediately under the urothelium they form a complex network and some terminals are even located within the basal layer of the urothelium (Gabella and Davis 1998; Wiseman et al. 2002). Close to the detrusor muscle both myelinated and unmyelinated fibers can be found. Some claim that in the mucosa only unmyelinated fibers are present (Fowler 2002; Wiseman et al. 2002), but others have reported on mucosal afferents with conduction velocities corresponding to myelinated fibers (Schalow and Lang 1989; Winter 1971).

Afferent nerve activity from bladder was investigated first by Evans in the cat (1936) and Talaat in the dog (1937) but it was only later the activity of single pelvic afferent fibers was related to detrusor muscle tension (Iggo, 1955). Since then, numerous studies have investigated the properties of bladder afferent fibers. The sensory receptors in the bladder exhibit no ongoing

resting activity, appear to be low threshold mechanoreceptors and are innervated by myelinated Aδ afferent fibers (Bahns et al. 1987; Habler et al. 1993a; Janig and Koltzenburg 1991). The response behavior of this type of receptors differs per units: the activation threshold ranges 6 to 38 cm H_2O[1] and the firing frequency increases in a graded manner with an increase in intravesical pressure, see example in Figure 5. The maximum frequency is however reached before the pressure reaches it's maximum level and is maintained during further increase in pressure by some units while others display a reduction in firing frequency after the maximum frequency has been reached (Habler et al. 1993b; Iggo 1955; Winter 1971). Although the relation between the activity of these afferents and both the intravesical pressure or calculated wall tension in the receptor fields is non-linear, the tension appears to offer a more precise relationship (Downie and Armour 1992).

There exists also a second type of receptors innervated by unmyelinated C afferent fibers who's function is less homogeneous and their characteristics depend on the animal model used (Morrison 1999). In the cat, these receptors have a high activation threshold and are unresponsive to mechanical stimuli such as bladder filling, but they do respond to chemical, noxious or cold stimuli (Fall et al. 1990; Habler et al. 1990). The same type was found to respond to distension in the rat bladder mucosa. They have also higher thresholds compared to the Aδ mechanoreceptors (40-55 cmH_2O), they do not respond during bladder contraction and they may also be sensitized by the chemical composition of the bladder content or inflammation of the bladder mucosa (Morrison 1999).

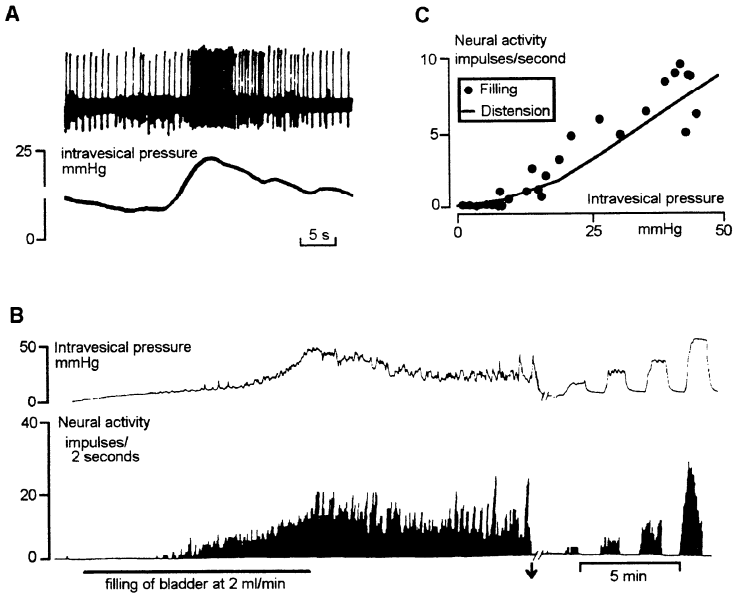

Fig. 5: Typical response from a myelinated pelvic nerve afferent single unit to slow filling and isotonic distension of the bladder (cat). **A**: Neural activity increases with an increase in intravesical pressure. **B**: Histogram of the impulse activity and intravesical pressure during slow filling (first part) and isotonic distension of the bladder (after bladder emptying, indicated by the arrow). **C**: Stimulus response function obtained for this particular afferent unit. Adapted from Habler et al. (1993a)

Nerve fibers are classified based to their function and conduction velocity (CV, see table 2). The CV of myelinated Aδ afferent fibers from the bladder is distributed over a broad range, but

[1] Conversion factor used: 1 mmHg = 13.6 mm H_2O.

there is a tendency of the slowest fibers to be associated with detrusor receptors (CV < 16 m/s), an overlapping but slightly faster group to be associated with mucosal receptors (CV = 18-22 m/s), and the fastest group to be associated with receptors located in the perivascular tissue (CV = 25-40 m/s). The unmyelinated fibers have a much lower CV than Aδ fibers. Afferent fibers with CV smaller than 2.5 m/s are generally considered to belong to the group of C fibers.

The traditional classification schemes are based on animal data but, because the CV's in animals are different than in human, Schalow suggested that they would be inappropriate for application in human studies and an alternative scheme for the human peripheral nervous system was developed (Schalow 1991; Schalow 1992; Schalow et al. 1995b; Schalow et al. 1995a). This scheme (figure 6) intents to provide a more detailed classification based on the group conduction velocity, group nerve fiber diameter and function of different sacral root afferent and efferent nerve fibers innervating the skin, bladder, anal canal and muscle. Different types of bladder receptors were characterized in detail during retrograde bladder filling. However, afferent activity evoked by bladder catheter pulling was identified as from one type of bladder receptor (M) and several other skin mechanoreceptors whereas activity from receptors in the urethra, such as the flow sensitive ones facilitating bladder emptying (section 4.3) was not identified separately. The latter could suggest that the group CV's of urethra afferents is similar to those from the skin mechanoreceptors and therefore responses were not distinguishable. Another limitation of the above scheme is given by the fact that the absolute numbers are specific for the sacral roots. Direct comparisons with CV data from pelvic or hypogastric nerves should be made cautiously because nerve fibers can conduct more slowly in the dorsal root than in the peripheral nerve (Waddell et al. 1989).

	Conduction Velocity [m/s]		Response threshold [cm H_2O]	Recording site	Receptor or stimulus type	Reference
	Unmyelinated	Myelinated				
Cat		25-40 18-22 < 16		PVN	Perivesicular tissue Mucosa Detrusor	(Winter 1971)
	0.5 – 1.5 1.0±0.5 1.4±0.6 0.8±0.3	10.3±6.1	6.8 – 24.5 41 – 68	S1/S2 S2	Electrical stimulation Bladder distension Chemical irritation	(Bahns et al. 1987) (Habler et al. 1990)
		1 - 8 2.5 – 15 2.6 – 12.5 3.2 – 15.3	5 - 30 < 30 < 25 0.6 - 3.1 2.8 - 24	PVN S2 S2 PVN	Bladder distension Chemical irritation Slow distension Fast distension	(Downie and Armour 1992) (Habler et al. 1993a) (Habler et al. 1993b) (Satchell and Vaughan 1994)
Rat	0.5 - 2.5	2.5 – 21 2.5 – 6		PVN	El. stimulation of L6 El. stimulation of S1	(Vera and Nadelhaft 1990)
	1.77±0.06 1.65±0.08	6.4±1.6 8.4±2.8	0 – 19.9 38.1 – 60.9	S1	Low threshold fibers High threshold fibers	(Sengupta and Gebhart 1994)
			10.2 – 20.4 4.1 – 8.8	HGN PVN		(Moss et al. 1997)
	0.5 – 1.4	2 – 10.6	6.7±8.6 12.0±11.0	L6		(Shea et al. 2000)
	0.5 – 1.15	1.35 – 8.8		L6/S1	In vitro	(Namasivayam et al. 1998)
Rh. monkey Chimpanzee		22 (7-47) 31 (13-43)		L7-S2 S1-4	El Stimulation PVN El Stimulation PVN	(Rockswold et al. 1980)
Human		37.5 – 47.5 30 – 37.5 10 – 17.5		S5	Stretch receptors (Over) stretch receptors Flow, touch, high pressure	(Schalow 1991; Schalow 1993; Schalow et al. 1995b)

Table 2. Group conduction velocities (CV) and response thresholds of bladder afferents as reported by different sources in the literature. Values reported as range or mean ± standard deviation (conversion applied in some cases from either standard error or pressure levels in mmHg). In the cat, fibers with CV's less than 2.5 m/s are considered unmyelinated (Habler et al. 1990). In the rat, fibers with CV's less than 1.3 m/s (Namasivayam et al. 1998) or 2.5 m/s (Sengupta and Gebhart 1994) are considered unmyelinated and the response threshold is estrous cycle sensitive (Shea et al. 2000). Abbreviations: PVN: pelvic nerve, HGN: hypogastric nerve, S1: first sacral nerve root, L6: sixth lumbar nerve root.

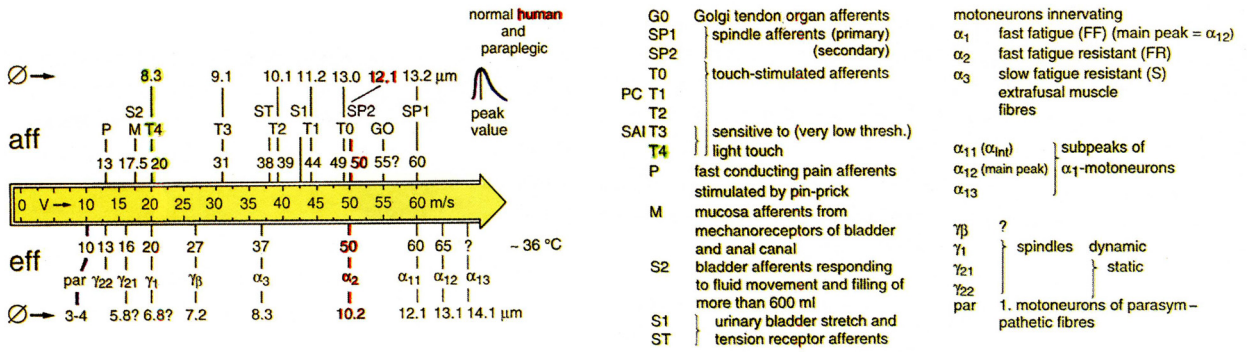

Fig. 6: Conduction velocities (CV) and nerve fiber diameters (Ø) of afferent and efferent sacral root nerve fibers from normal humans and patients with traumatic spinal cord lesion according to the classification scheme by Schalow et al. (1995b).

6.3 Recording whole nerve activity from the bladder

In the studies mentioned previously, stimulus-response functions were obtained based on activity recorded from small nerve filament preparations. The nerves were isolated from the general body fluids by surrounding them with paraffin or mineral oil to restrict the extracellular space and nerve activity was recorded using wire or hook electrodes. However, this technique is not suitable for chronic application because of the damage inflicted to the nerve fibers during dissection and the insulating media used (mineral/paraffin oil) are not biocompatible. Furthermore, the obtained information is specific for the type of receptors recorded from as well as method of stimulation and location of the receptor in the bladder. Some studies have shown that a close relationship between afferent activity and changes in intravesical pressure can still be observed when the activity of many units is summed (Bahns et al. 1986; Winter 1971). Others found the activity recorded from the whole postganglionic bladder nerves to be proportional to bladder pressure (Le Feber et al. 1997). Thus, aggregate information from different types of sensory receptors from the bladder can be obtained by recording the activity from whole, intact nerves.

Alternatively to using wire or hook electrodes, whole nerve activity can also be recorded using nerve cuff electrode. A cuff electrode consists of a piece of electrically insulating material (usually a medical grade silastic) with one or more recording contacts on the inside. When installed around the nerve, it restricts the extra cellular space and increases the amplitude of the extracellular nerve action potentials inside the cuff in a way similar to the paraffin or mineral oil in the above acute experiments (Stein et al. 1975). Other advantages of using cuff electrodes are that the extraneural application does not disrupt the integrity of nerve and most interference signals generated external to the cuff can be rejected depending on the electrode contact configuration used (Hoffer 1990; Stein et al. 1975; Stein et al. 1977). Since Hoffer and Sinkjaer (1986) first proposed that nerve cuff recording electrodes implanted on cutaneous nerves could be used to render a feedback signal proportional to skin contact force for close-loop control of FES, cuffs electrodes have been the foremost used electrode in neuroprostheses research.

Jezernik and co-workers used cuff electrodes to record nerve activity from the pelvic nerve, and first (S1) to third (S3) extradural sacral roots in pigs (Jezernik et al. 2000) and cats (Jezernik et al. 2001) during slow filling and rapid distension of the bladder. In the pigs, the most consistent nerve responses were recorded from the pelvic nerve and the S3 sacral root during the rapid bolus infusions. A nerve response during slow bladder filling was recorded in only less than half of the pigs. Based on these results, a linear model for the recorded nerve activity as a function of the bladder wall tension was proposed. This new model expanded the proportional model by (Le Feber et al. 1997) to a first order model by including tonic changes in bladder tension as well as phasic changes (time derivative of the tonic term) (Jezernik et al. 2000).

Slow bladder filling led only in the cat study to quasi-periodic bladder contractions during which the nerve activity from mainly S1 increased and correlated with the bladder pressure. Furthermore, applying electrical stimulation of the extradural S1 sacral root was only able to modulate ongoing bladder contractions in one cat while contractions were terminated when applied to the S1 dorsal root in another animal.

6.4 Chronic monitoring of nerve activity from the bladder

All acute animal studies described so far involved invasive surgical procedures that require the use of general anesthesia. However, many anesthetics have an adverse affect on the reflex pathways controlling bladder activity (Matsuura and Downie 2000; Rudy et al. 1991). Depending on the doses and types of anesthetics used, they can completely obliterate reflex bladder activity, as for example seen in the pig study by Jezernik et al. (2000). The best way to overcome adverse effects of anesthesia on the neural pathways controlling the bladder is of course not to use anesthesia at all. For this the experimental set-up has to be transformed into a chronic setting, in which the recording electrode is implanted by a separate surgical procedure such that nerve recordings can be made during natural bladder activity in the conscious animal in later experiments.

The pelvic nerve would be the preferred site for chronic implantation based on the high correlation between the pelvic nerve recordings and bladder pressure (Jezernik et al. 2000). However, a preliminary study where cuff electrodes were chronically implanted on the pelvic nerve proved unsuccessful (Jezernik 2000). After a period of approximately one year the cuff electrodes had migrated and histology showed no myelinated nerve fibers inside the cuffs. A more optimal site is at the level of the sacral nerve roots because the space within the spinal canal facilitates mechanical stable electrode positioning and the intraspinal course of the sacral roots is sufficiently long (Rijkhoff et al. 1997).

Nerve cuff electrodes have been proven suitable for chronic use, both in animal (Hoffer 1990; Popovic et al. 1993; Stein et al. 1975; Stein et al. 1977; Struijk et al. 1999) as well as in human applications (Haugland and Sinkjaer 1995; Slot et al. 1997). Much is therefore already known from these studies about the impact of surgical manipulations and the electrode itself on the integrity of the neural structure and the influences it can have on the nerve activity recorded. Previous findings obtained from distal peripheral nerves could possibly be projected to the sacral roots but the unique implantation location (within the spinal column) and the composition of nerve roots themselves may give a different the outcome. Cuff electrodes were implanted on the sacral roots in a few human studies, but their application has been limited to electrical stimulation (Hohenfellner et al. 1998; Matzel et al. 2001) and there is no experience with chronic recording from those nerves.

In the above mentioned recording studies using chronic implanted cuff electrodes, the electrode wires were taken out either percutaneously or through a subcutaneous a connector (accessed by a small surgical procedure for each recording session). These are no good long-term solutions suitable for clinical applications as mechanical stress inserted on the percutaneous wires can easily inflict nerve damage and the permanent breakage of the skin barrier pose unacceptable problems of cosmesis, maintenance and infection (Marsolais and Kobetic 1986; Yarkony et al. 1992). To avoid this, a telemetry system for long-term neural recording in animal studies as well as human clinical applications was developed recently (Donaldson et al. 2003). This system consists of an implantable device (telemeter) that amplifies the signal recorded from the cuff electrode and transmits it from within the body to an external drive box. The telemeter (figure 7) is powered and the amplified signals are transmitted to and from the implant by induction.

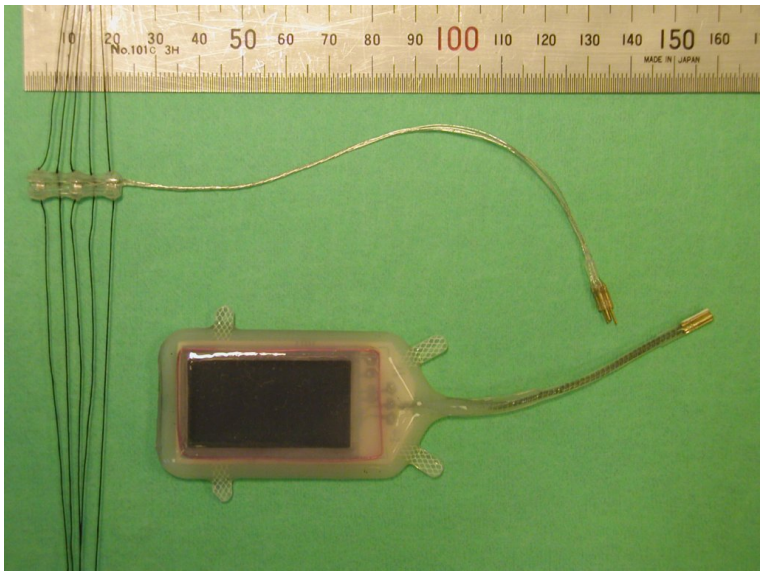

Fig. 7: Tools for long-term recording of whole nerve activity: a tripolar nerve cuff electrode and an implantable telemeter as used in the studies described in this thesis. The sutures attached externally to the cuff are first used to facilitate installation and afterwards used to keep the cuff closed.

6.5 From animal model to human

The technology for long-term interfacing with the peripheral nervous system has reached such a level that it is ready for transfer from animal models to human applications (Sinkjaer et al. 1999; Sinkjaer et al. 2003). The implant of cuff electrodes in human for recording of nerve activity from the natural sensors has been pioneered at the Center for Sensory-Motor Interaction at Aalborg University, and already resulted in neural prosthesis systems to correct foot drop (Hansen et al. 2002; Haugland and Sinkjaer 1995) and restore hand grasp (Haugland and Sinkjaer 1995; Inmann et al. 2001).

The possibility for investigating the feasibility of recording nerve activity from the bladder in human is limited to so-called intraoperative monitoring when a patient is undergoing surgery as part of a treatment and access to the nerves of interest is available. Bladder afferent nerve activity was first recorded in humans by Schalow and Lang (1986, 1987) from small sacral nerve root filaments in patients who were implanted with a sacral anterior root stimulator for control of bladder and bowel (Brindley et al. 1986). In a few other studies sacral root compound nerve action potentials were recorded in response to electrical stimulation of the dorsal penile or clitoral nerves in patients with cerebral palsy undergoing selective posterior rhizotomy to treat spasticity (Deletis et al. 1992; Huang et al. 1997). However, whether sensory nerve activity from the bladder can be recorded from cuff electrodes placed on the intact sacral nerve roots in human is still unknown.

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Chapter 2

Chronic implantation of a cuff electrode for recording of sacral root nerve signals in pigs.

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To be submitted

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Abstract

The purpose of this study was to investigate the feasibility of implanting a cuff electrode chronically on the sacral nerve roots for recording of afferent nerve signals. These signals could be used as feedback for an implantable neuroprosthesis to control the overactive bladder. Cuff electrodes were implanted in seven female Göttingen mini pigs on the extradural sacral nerve roots S1 (n=1), S2 (n=4) and S3 (n=3), and connected to a subcutaneously implanted telemetric device. Nerve signals were evoked by electrical stimulation of the clitoral nerve and mechanical stimulation of the relevant dermatome. Nerve signals were recorded in all animals on the day of implantation, and in five animals at the first follow-up experiment, 7 to 19 days after implantation. The most successful implant lasted 383 days. Reasons for terminating an implant were puncture of the skin, infection, suspected brain damage after anesthesia, and no nerve responses. Histological results showed signs of neural damage in 4 of 5 examined animals. The results show that cuff electrodes can be implanted chronically for recording of sacral root afferent nerve signals, but also that when nerve damage is inflicted during implantation or later on, this will be clearly reflected in the recorded nerve signals.

1 Introduction

Sensory signals recorded from peripheral nerves have become a realistic alternative to artificial sensors for feedback control in neural prostheses (Haugland and Sinkjaer 2000; Hoffer et al. 1996; Johnson et al. 1995). Natural sensory feedback from cutaneous receptors has been employed in neural prostheses to correct foot drop (Haugland and Sinkjaer 1995) and restore hand grasp (Inmann et al. 2001). Signals recorded from muscle receptors have been used as feedback signals in a functional electrical stimulation (FES) control of joint position in an animal model (Yoshida and Horch 1996). Based on earlier reports that bladder sensory activity increased with increasing tension in the bladder wall (Habler et al. 1993; Winter 1971), the feasibility of using these signals as feedback for an implantable neuroprosthesis to control the overactive bladder has also been explored recently (Jezernik 2000)

Several acute studies have demonstrated that afferent nerve signals related to mechanical bladder activity can be recorded using cuff electrodes placed on the pelvic nerves and (dorsal) sacral nerve roots in pig (Jezernik et al. 2000), cat (Jezernik et al. 2001), and human (Kurstjens et al. 2005). Although bladder afferent activity was recorded in all these studies, only in the cat it could be recorded during bladder contractions induced by slow saline infusion. Possible causes for this may include the effect of anesthesia, urethal ligation (used in the pig), or the different physiology of the different species (Jezernik 2000; Klevmark 1980). The reflex pathways mediating bladder contractions are indeed affected adversely by anesthetics (Matsuura and Downie 2000; Rudy et al. 1991). These findings indicate the necessity to avoid the use of anesthetics when recording nerve signals related to such reflex activity, i.e. to perform conscious recordings.

For recording of neural activity in the unanesthetized state, chronic implantation of electrodes is needed. The best correlation between bladder pressure and neural activity is obtained when recording from the pelvic nerves (Jezernik et al. 2000), but implantation on these nerves is surgically difficult and they are easily damaged during cuff electrode placement. A more optimal site for electrode application is the sacral nerve roots (Rijkhoff et al. 1997). The space within the spinal column facilitates mechanically stable electrode positioning and the long intra-spinal course of the sacral roots simplifies application of electrodes. The sacral nerve roots have therefore been a favourite site for electrode application for control of bladder and urethral sphincter (Brindley 1994; Hohenfellner et al. 1998; Tanagho and Schmidt 1988), and anal sphincter (Matzel et al. 2001). Chronic application of electrodes on the extra and intra-dural sacral roots has so far been limited solely to electrical stimulation and no studies have reported before their use for recording of electroneurographic (ENG) signals.

When using chronically implanted cuff electrodes for recording, the ENG signals are mainly influenced by three factors: electrical properties of the electrode, the tissue inside and around the cuff, and changes in the neural structure (Thomsen 1998). In growth of connective tissue increases the impedance between contacts (Stein et al. 1978; Grill and Mortimer 1994; Malek and Mark 1989) and affects the amplitude of the recorded signal (Stein et al. 1975; Struijk et al. 1999). Surgical manipulation of the nerves, as well as the cuff electrode itself, may inflict axonal damage. Compression and angulation neuropathy will most severely affect the larger diameter axons and reduces the axonal conduction velocity (Gillespie and Stein 1983; Sunderland 1978). Cuff electrodes implanted on peripheral nerves have shown to induce a small loss of large diameter myelinated axons (Stein et al. 1977), but a subsequent regeneration has also been reported (Larsen et al. 1998).

The purpose of the present study was to investigate the feasibility and safety of implanting a cuff electrode on the extradural sacral nerve root for long-term recording of afferent nerve signals. Following implantation in mini pigs, the state of the neural interface was assessed periodically on non-invasive follow-up experiments by recording the neural responses to natural and artificial stimulation of sacral afferent pathways. After termination of the implant, the implanted nerve was removed and a histological examination performed.

2 Materials and Methods

2.1 Implanted devices

Split silicone (MED-117 Adhesive Silicone Type A, NuSil Silicone Technology, USA) nerve cuffs were fabricated according to (Haugland 1996). The cuffs were 15 mm in length, had an inner diameter of 1.8-3.0 mm, and had three circular contacts made of either Teflon-coated multi-stranded stainless steel wire (AS 634 Cooner Wire Co., USA) or platinum foil (25 µm thick, 1 mm wide). A reference contact made out of the same material was mounted on the outside of the cuff.

The electrode contacts were connected in a quasi-tripolar configuration and nerve signals were recorded using to a telemetric system specially designed for long-term neural recording in animals and humans (Donaldson et al. 2003). This system included an implantable telemeter and an external receiver/drive unit to supply power through inductive coupling. The cuff electrode and telemeter were cleaned in an ultrasonic cleaner prior to implantation, first in 99.9% ethanol and then in de-mineralized water, before autoclave sterilization.

2.2 Implant procedure

The nerve cuff electrode and telemeter were implanted under general anesthesia and sterile conditions in seven Göttingen mini-pigs (28-45 kg). Intramuscular injection of Dormicum® (Midazolam) and Ketalar® (Ketamine) were used to sedate the pigs in order to obtain intravenous access. Intubation was performed after intravenous administration of Hypnomidate® (Etomidate) and general anesthesia was maintained by 1.5-2% Isoflurane. Antibiotic drugs (Ciprofloxacin 2 mg/ml, 200 ml I.V. and Gentamycin 40 mg/ml 2 ml I.V.) were administered twice during the implant procedures: 30 minutes before operation start and 30 minutes before closure of the incision.

A sacral dorsal laminectomy was performed to gain access to the extradural sacral nerve roots. The nerve roots were identified anatomically and by the response to electrical stimulation. A cuff electrode, with inner diameter about 20% larger than indicated by the nerve (Hoffer 1990), was placed on the extradural sacral nerve root that showed the largest increase in bladder pressure in response to electrical stimulation with a hook electrode, and the impedance of each contact was measured (1 kHz sine wave, ±90 nA) with respect to the reference contact. The signal recorded from the cuff was then checked to contain afferent signals by mechanically stimulating the appropriate dermatome.

The wires from the cuff electrode were routed subcutaneously from the distal end of the cuff and with a small loop to relieve stress, to the telemeter which was placed in the subcutaneous fatty tissue layer, between 1 and 2 cm deep, just lateral to the spine and approximately 5 cm cranial to the pelvis.

2.3 Experimental procedure

Follow-up experiments started generally two weeks after implantation and were repeated periodically. Experiments were initially performed under general anesthesia (Isoflurane 1.5%, induced as described in implantation procedure), but conscious recordings have also been performed with 3 of the 7 animals. A urine dipstick test (Multistix®, Bayer Co., USA) was performed before each experiment to test the pig for cystitis.

Responses of sacral afferents to artificial and natural stimulation were recorded to assess the state of the neural interface. Artificial stimulation was applied by transcutaneous electrical stimulation of the clitoral nerve. A handheld bipolar surface electrode (Dantec-Metronic) was placed on the labium major, and rectangular constant current pulses of 0.2 ms duration, 1-40 mA in amplitude, and with stimulation frequency of 6-8 Hz, were used to elicit sensory nerve compound action potentials (CAPs). Natural stimulation was applied by manually tapping the appropriate dermatome for maximum response.

To activate the telemeter, the transmitter was placed over the location where it was implanted and the best position was found for optimal power transfer and signal coupling. The total gain of the telemetric system was between 63000-80000, depending on the telemeter/control-box combination. The signals received from the telemetric system were additionally filtered (200 Hz – 4 kHz) with either an analog filter (Krohn-Hite, model 3103) or a digital filter module (Versa-Filter, Signal Processing Solutions, USA), before sampling (at 20 kHz) and storage on a PC.

2.4 Signal processing

Individual CAP responses to the electrical stimulation were averaged to improve the SNR. The amplitude was measured peak-to-peak (pp) and the latency as the time to the first positive peak, reflecting the conduction velocity of fast conducting fibers (Buchthal and Rosenfalck 1966). These amplitude and latency measurements have proven to be the most reproducible from day to day (Davis et al. 1978). Measurement of the CAP parameters was performed using data from a time window between 1.5 to 6 ms after stimulation to exclude stimulation artifacts ($t < 1.5$ ms) and reflex responses ($t > 6$ ms).

All ENG responses to mechanical stimulation were additionally high-pass filtered off-line using a zero phase eight-order Butterworth filter at 800 Hz (Gordon et al. 1980; Popovic et al. 1993) because a large increase in the level of background neural and muscular activity was often observed during the awake experiments.

The signal-to-noise ratio (SNR) and the median frequency of the ENG power spectrum density (PSD) were calculated from mechanically evoked nerve responses. The SNR of the recorded cutaneous ENG was defined as the ratio between the variance of the recorded ENG signal with and without mechanical stimulation, when only noise and background neural activity was assumed to be present. For each skin tap, the variance was calculated for a 40 ms time window centered on the peak of neural activity. This window length was chosen because it represented well the duration of peak neural activity and contained sufficient data points for the frequency analysis. The level of background activity (BGN) was obtained by dividing one second of signal into bins of 40 ms duration, and calculating the average of the variances of each of these data bins. The PSD's of both the ENG during cutaneous stimulation and the background noise signals were calculated (800 point FFT for 25 Hz resolution) using the same data bins as used to calculate the SNR. The average BGN spectrum was subtracted from the ENG spectrum. The median frequency was calculated for each PSD and then averaged for the total number of taps applied.

2.5 Nerve histology

After the conclusion of the implant period, the pigs were anesthetized and intubated (see 'Implant procedure'). The subcutaneous pocket was opened and the telemeter disconnected and removed. Thereafter, the cuff electrode, underlying nerve and surrounding tissue were excised and fixed in 4% formaldehyde buffer (pH 7.0) until histological processing. Tissue blocks were cut at the cuff level, proximal and distal to the cuff, depending on the size of the excised tissue specimen. The tissue blocks were dehydrated and imbedded in paraffin wax. Sections (4 μm thin) were cut perpendicular to the longitudinal axis of the nerve, re-hydrated and stained with hematoxylin and eosin. The histology of the nerve tissue was evaluated by light microscopic examination with regards to signs of inflammation and neural damage.

2.6 Statistical analysis

A one-way ANOVA test was used to investigate the effect of implantation time on the level of background activity, SNR, median frequency, and Dunnett's test was used for post-hoc analysis. The Wilcoxon signed rank test was used to compare the electrode impedance measured at implantation versus explantation, and the Student's-test was used to compare the levels of background activity at different experiment dates. An error probability of less then 5% ($p<0.05$) was considered statistically significant.

3 Results

In the seven implanted pigs, cuff electrodes were placed on extradural sacral nerve roots with a distribution as shown in table 1. A cuff diameter much larger than indicated by the nerve root itself was used in three pigs to accommodate the dorsal root ganglion. The average impedance of the electrode contacts at implantation was 1571 Ohm (range: 900 – 2600 Ohm), but was significantly larger ($p<0.007$, $n=15$) at the day of explantation (2580 Ohm, range: 1400 – 6400 Ohm).

Pig	Sacral root	Cuff diameter [mm]	Implant duration [days]	Reason for termination
1	S2, L*	2.6	19	Brain damage
2	S2, L & R**	2.2 & 3.0	49	Infection, telemeter malfunction
3	S1, L	2.2	383	Skin puncture
4	S3, L	2.6	35	No nerve responses
5	S3, R	1.8	30	No nerve responses
6	S2, L	3.0	42	Nerve damage, constipation
7	S3, R	2.2	189	No nerve responses

Table 1, Implantation data. *L, R = Left, Right. **Bilateral implantation.

3.1 Individual implantations

The duration of implantation and the reason for termination varied between pigs, as summarized in table 1. ENG responses were absent in two pigs at the first follow up examination while the most successful implant lasted 383 days.

Intubation of pig 1 at the first follow-up went troublesome, and a possible lack of oxygen might have caused brain damage as the pig had difficulties to breath spontaneuosly when recovering from anesthesia. The pig was therefore sacrificed after the experiment although good nerve responses were recorded and there were no other implant related problems.

In the bilaterally implanted pig 2, an infection developed in the short-term postoperative period. An edema was clearly visible at the location of the left telemeter after 14 days of implantation and later also around the right telemeter. On day 35, only an oscillatory signal was received from the right telemeter indicated a malfunctioning device, and the pig was also diagnosed with cystitis (was treated with Cefuroxim®). The implant was then terminated after 49 days because both telemeters had punctured the skin.

The telemeter implanted in pig 3 started to malfunction on day 28. Cutaneous ENG responses were however present on day 59 when the telemeter was removed and an external amplifier (ADT-1, Micro Probe, Inc.) connected to the cuff. Following this, a new telemeter was implanted 84 days after initial surgery. The telemeter eventually punctured the skin, and the implant had to be terminated after 383 days although good nerve responses were recorded.

During the first follow-up of pig 4 and pig 5, after respectively 21 and 30 days, no nerve responses were recorded although both pigs demonstrated normal behaviour, and there where no signs of nerve damage. On removal of the telemeters, respectively 35 and 30 days after implant, both devices functioned properly, but two broken lead wires were found in pig 4.

Pig 6 was diagnosed with diarrhea after 15 days of implantation, but it had recovered when checked again on day 36. The pig was found ill again on day 42: the urine was more viscous than normal and tested positive for blood and proteins. Signs of nerve damage were also demonstrated by almost no responses to touching the dermatome and walking with a limp. An autopsy after the last experiment revealed that the pig had suffered from constipation.

The lack of response to pin picking the dermatome with a needle on day 21 of implantation in pig 7 indicated nerve damage as applying the same procedure on contralateral dermatome resulted in a strong avoidance response from the pig. Because a small cutaneous ENG response recorded on day 35 could suggest a possible regeneration of nerve fibers, the pig was given tree months to allow regeneration to continue, but although on day 133 a withdrawal responses to contact with the dermatome was present again, the implant was terminated after 189 days because no nerve responses were recorded.

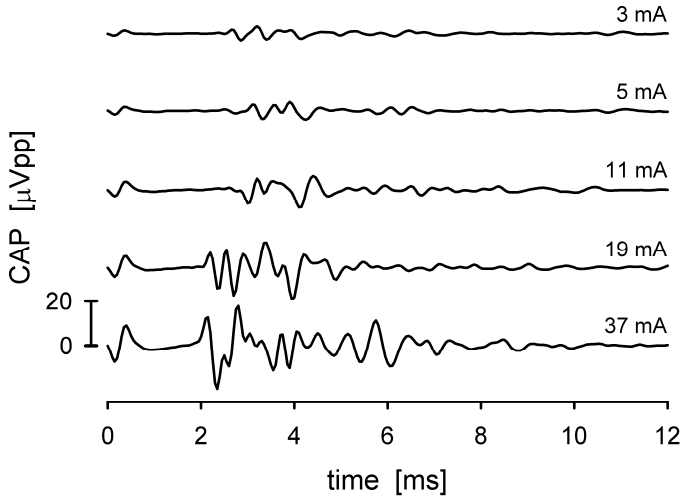

Fig. 1: CAP responses recorded at the day of implantation in pig 3 (average of at least 82 stimuli). Note the stimulation artefact at time = 0 ms.

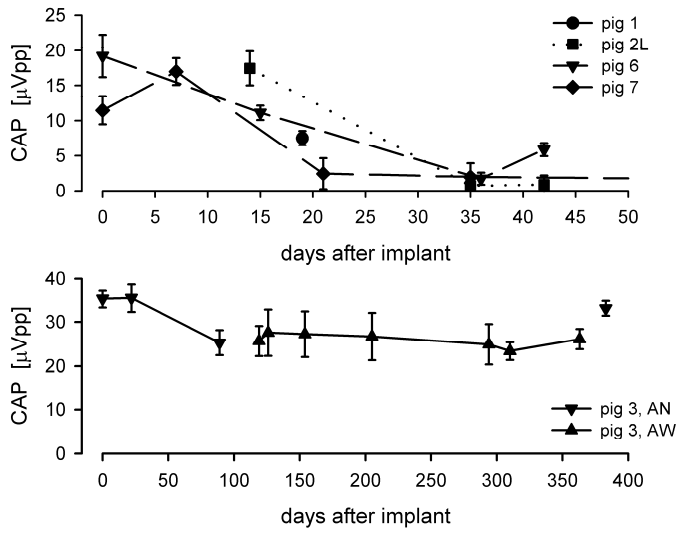

Fig. 2: Amplitude of averaged CAP responses recorded during supra-maximal electrical stimulation on follow-up experiments in pigs 1, 2, 6, and 7 (upper graph), and in pig 3 (lower graph). AN indicates anesthetized and AW indicates awake experiments for pig 3.

3.2 Sensory compound action potentials

Amplitude of sensory compound action potentials

Electrical stimulation of the clitoral nerve was not performed on the day of implantation in pigs 1, 2 and 5. In the other 4 pigs, maximum CAP responses of 11.5±2.0 µVpp (pig 7, n=36) to 35.3±2.0 µVpp (pig 3, n=83) were recorded when applying a stimulation current of respectively 28.9 mA and 37.1 mA. Figure 1 shows averaged CAPs, recorded on the day of implantation in pig 3 as the stimulation current was increased from 3 to 37 mA. The CAP amplitude was 6.3 µVpp when stimulating with 3 mA, and increased with increasing stimulation current until a maximum CAP amplitude of 37.0 µVpp was reached at 37 mA. As more and more fibers are

activated, their contribution did not only increase the amplitude but also altered the shape and decreased the latency of the compound response.

The maximum amplitudes of the CAP responses recorded at follow-up experiments are shown in figure 2. The results from pig 3 are plotted in a separate graph (2B) because the implantation time was much longer than in the other pigs. CAP responses were present in 5 out of 7 pigs at the first follow up, 7 to 19 days after implantation. At the third follow up experiment, the CAP amplitudes were reduced in 6 of 7 pigs. Pig 6 showed however signs of recovery when an increase in CAP amplitude was recorded on the day of explantation (day 42). The amplitude of the CAP responses in pig 3 had decreased by approximately 30% on day 89, but remained stable over time for the rest of the implantation period. Because of the implant proved stable, all remaining follow up experiments in pig 3 were performed while the animal was awake.

Latencies of sensory compound action potentials

The latency of the averaged CAP responses decreased when increasing the stimulation current. On the day of implantation in pig 3, the onset latency decreased from 2.65 ms when stimulating with 3 mA, to 2.15 ms when stimulating with 37 mA (see Figure 2). The CAP onset latencies for maximal stimulation on the day of implantation in the other pigs ranged from 1.65 ms in pig 4 (I = 35 mA) to 2.10 ms in pig 6 (I = 36 mA).

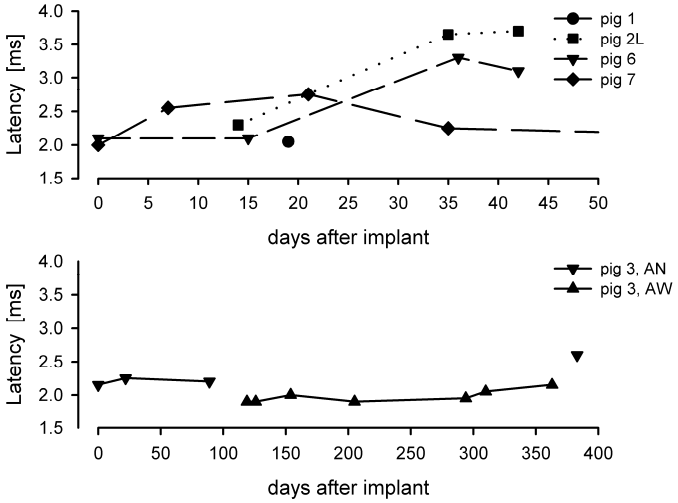

Fig. 3: Latency of the averaged CAP responses to supra-maximal electrical stimulation during the total implantation period in pigs 1, 2, 6, and 7 (upper graph), and in pig 3 (lower graph). For pig 3 the line is interrupted to indicate transitions between awake and anesthetized experiments.

Figure 3 shows the latencies of CAPs recorded during the implantation periods. The latencies of pigs 2, 6, and 7 increased with time (Figure 3A). The decrease on day 35 (to 2.25 ms) in pig 7 could be a sign of recovery, as it continued to 2.05 ms on day 91 (not shown). Pig 6 also showed a small recovery from 3.30 ms on day 36 to 3.10 ms on day 42. The latency in pig 3 (Figure 3B) remained relatively constant at the first two follow-up experiments, but suddenly decreased afterwards. This change coincided with the switch from anesthetized (0-89 days after implantation, and explantation on day 382) to conscious experiments (all other days in between) and was the same in both cases (0.45 ms).

3.3 Cutaneous nerve signals

Nerve responses to mechanically stimulating the dermatome were recorded on the day of implantation in all pigs. Tapping the skin evoked a distinct neural response. A typical example of the neural response recorded during tapping is shown in figure 4. The signal was recorded after 19 days of implantation in pig 1.

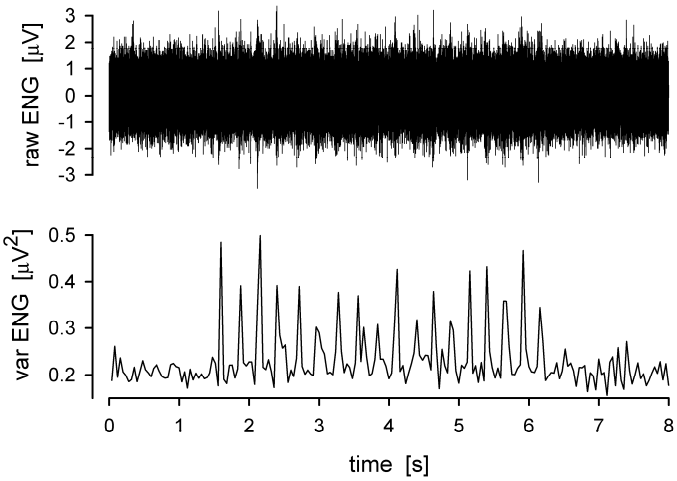

Fig. 4: Upper graph: raw nerve signal recorded during mechanical stimulation of the dermatome by applying a series skin taps to pig 1 on day 19 of implantation. Lower graph: variance of every 40 ms time bin for the data shown in the upper graph, demonstrating the presence of nerve responses with amplitude that is the same or lower than the noise.

The upper graph of figure 4 shows the raw nerve signal. The level of background activity (BGN) was around 2 μV (peak). The neural activity evoked by each skin tap is however hardly distinguishable. The lower graph of figure 4 shows that, to detect the presence of neural activity, a better separation between BGN and ENG can be obtained by calculating the variance of the signal per time bin. Bursts of neural activity, with variance peaks ranging 0.3 μV^2 to 0.5 μV^2 as the force and location were not exactly the same for each tap, now clearly stand out from the approximately 0.2 μV^2 background activity. The averaged SNR for the cutaneous ENG shown in Figure 4 was 1.01±0.29 (18 peaks).

Signal-to-Noise Ratios

The averaged SNR from the cutaneous response during skin tapping on the days of implantation ranged from 3.59±0.82 (n=65) in pig 4 to 15.45±6.27 (n=13) in pig 3. This was significantly larger than during the rest of the implantation period for all pigs, except on the day of explantation in pig 3. Figure 5 shows the SNR values that were obtained during the implantation period in all pigs.

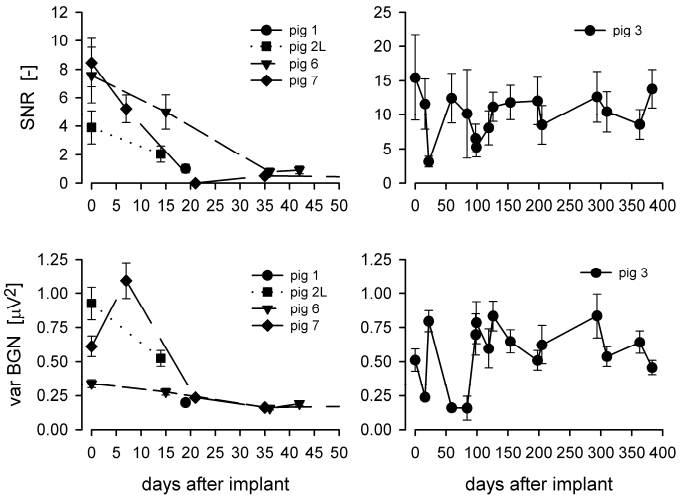

Fig. 5: Average SNR of the peak neural activity evoked by means of mechanical stimulation of the dermatome (upper two graphs), and levels of background activity (lower two graph) throughout the implantation period in pig 1, 2, 6, and 7 (left two graphs) and in pig 3 (right two graphs). Because of malfunction of the right implant in pig 2, only results from the left implant are shown.

The two upper graphs in Figure 5 show that cutaneous ENG responses during the full implantation period were only recorded in pig 3. On day 22 of implantation in pig 3, a sudden drop in SNR can be seen while the background activity was higher on the same day (Figure 5, lower right graph). After implanting a new telemeter on day 84, the averaged noise level did not change significantly ($p=0.905$) compared to the noise level recorded with the external amplifier on day 59, but when the following experiment was performed with the animal awake, a significant increase in background activity was observed ($p<0.001$). There was a visible tendency of an increased level of background activity on all following awake experiments. When grouping the levels of BGN for anesthetized and awake experiments, the averaged BGN level was significantly higher during the awake experiments than during the anesthetized experiments ($p=0.013$). The SNR of cutaneous responses in pig 6 were reduced to a minimum on day 36, but had slightly improved one week later. Recovery was also seen in pig 7 after 35 days, but the SNR did not improve afterwards.

Frequency response

Figure 6 shows the averaged power spectral density (PSD) of the peak cutaneous ENG recorded during skin tapping on the day of implantation in pig 6 (upper graph), and on day 35 when the amplitude of ENG responses was the smallest.

Most of the signal power was present between 1 and 3 kHz, and when comparing both spectrograms, it can be seen that on day 35 not only the total amount of signal energy was strongly reduced, but also that frequencies above 2 kHz were affected more than lower frequencies. This was expressed by a significant decrease in median frequency from 1936±131 ($n = 65$) to 1811±206 Hz ($n = 65$, $P<0.001$).

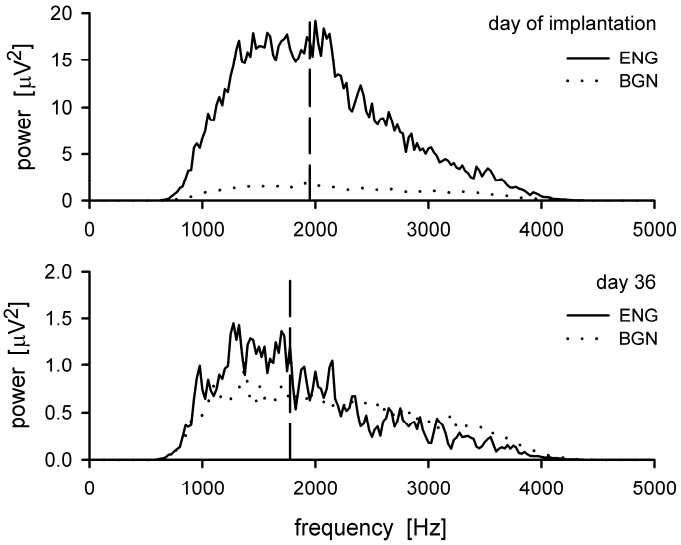

Fig. 6: Average PSD of cutaneous ENG responses (n=65) on the day of implantation in pig 6 (upper graph), and on day 36 when the lowest responses were recorded (lower graph). When comparing day 36 with implantation, the median frequency (vertical dashed line) was significantly decreased from 1936±131 Hz to 1811±206 Hz.

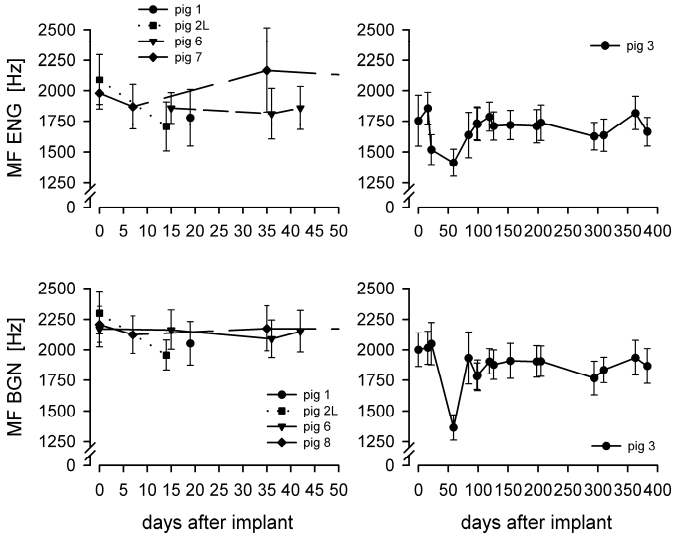

Fig. 7: Median frequency (MF) of cutaneous ENG responses (upper two graphs) and levels of background activity (lower two graph) throughout the implantation period in pig 1, 2, 6, and 7 (left two graphs) and in pig 3 (right two graphs). Because of malfunction of the right implant in pig 2, only results from the left implant are shown.

The average values for the median frequency of the ENG and BGN recorded during the implantation period in all pigs are plotted in figure 7. The reduction in signal amplitudes recorded during the follow-up experiments was also reflected in the frequency spectra. In pig 2, the median frequency of both ENG and BGN had decreased significantly ($p<0.001$) after 14 days. The median frequency of ENG responses recorded 16 days after implantation in pig 3 was slightly higher, but when measured again after 22 days of implantation, it was significantly reduced. On this day, the SNR and level of BGN were also found significantly changed (see

previous section 3.2.1), possibly because of an approaching malfunction of the telemeter (which occurred within the next few days). After 59 days, the median frequency and effective bandwidth of both ENG and BGN were significantly lower (see Figure 7, upper and lower right graphs). Recordings were made that day using the external amplifier. By day 98, all the latter parameters had regained levels that were not significantly different anymore compared to the values at implantation. Only the median frequency of the background activity remained significantly lower for the rest of the implantation period. The median frequency of ENG responses was significantly reduced at all follow-up experiments in pig 6 when compared to the day of implantation, whereas the changes in MF in pig 7 were not significant.

3.4 Histology

Nerve sections were taken from 5 of the 7 implanted pigs. Sections taken at the cuff level showed typically a layer of encapsulation tissue around the nerve (Figures 8 and 9), indicating a chronic inflammatory reaction, but no infection was found. The thickness and composition of this encapsulation tissue varied across animals, but it was generally divided in two zones: the inner part of the layer of encapsulation tissue consisted mainly of connective tissue and the outer part contained inflammatory cells, including macrophage, granulocytes and lymphocytes.

Nerve sections from pig 3 showed not only a strong chronic inflammatory response around the nerve but also some chronic inflammatory response within the nerve, but no signs of degeneration (Figure 8). Sections at the cuff level showed a large number of neurons and locally some longitudinal transected fibers, indicating that the cuff had been placed very close to or directly around the ganglion. This ganglion structure was also seen in nerve sections made at cuff level in two more animals, confirming the observations made during the implant procedure.

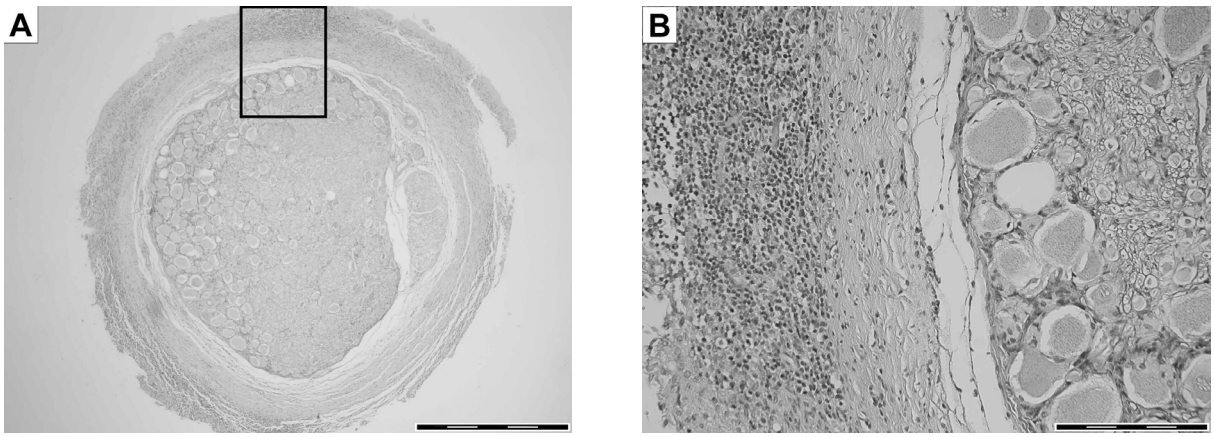

Fig. 8: Transverse nerve section at the level of the cuff in pig 3. (A) Low-power view (bar = 1 mm), showing the connective tissue encapsulation surrounding the nerve inside the cuff. (B) Higher-power view (90 degrees rotated, bar = 0.2 mm) of the area indicated in (A), showing on the right a part of the ganglion structure with neurons and normal looking myelinated fibers, in the middle the band of connective tissue, and on the left the large amount of inflammatory cells at the nerve cuff interface.

Histological changes could be observed in nerve sections from pig 2, 5, and 6 (30–49 days of implantation), but mainly in sections made around the proximal and distal ends of the cuff electrodes. The most prominent histological changes occurred at the cuff edge where the lead wires exit the cuff. Small, non-myelinated fibers were mostly intact but both small and large myelinated fibers showed degeneration of axons and myelin, and the connective tissue layer separating the fascicles was markedly thickened (see arrow in Figure 9d). Signs of regeneration were found in nerve sections from pig 2, but sections made distal to the cuff in pig 7 showed a completely degenerated and fibrosed nerve.

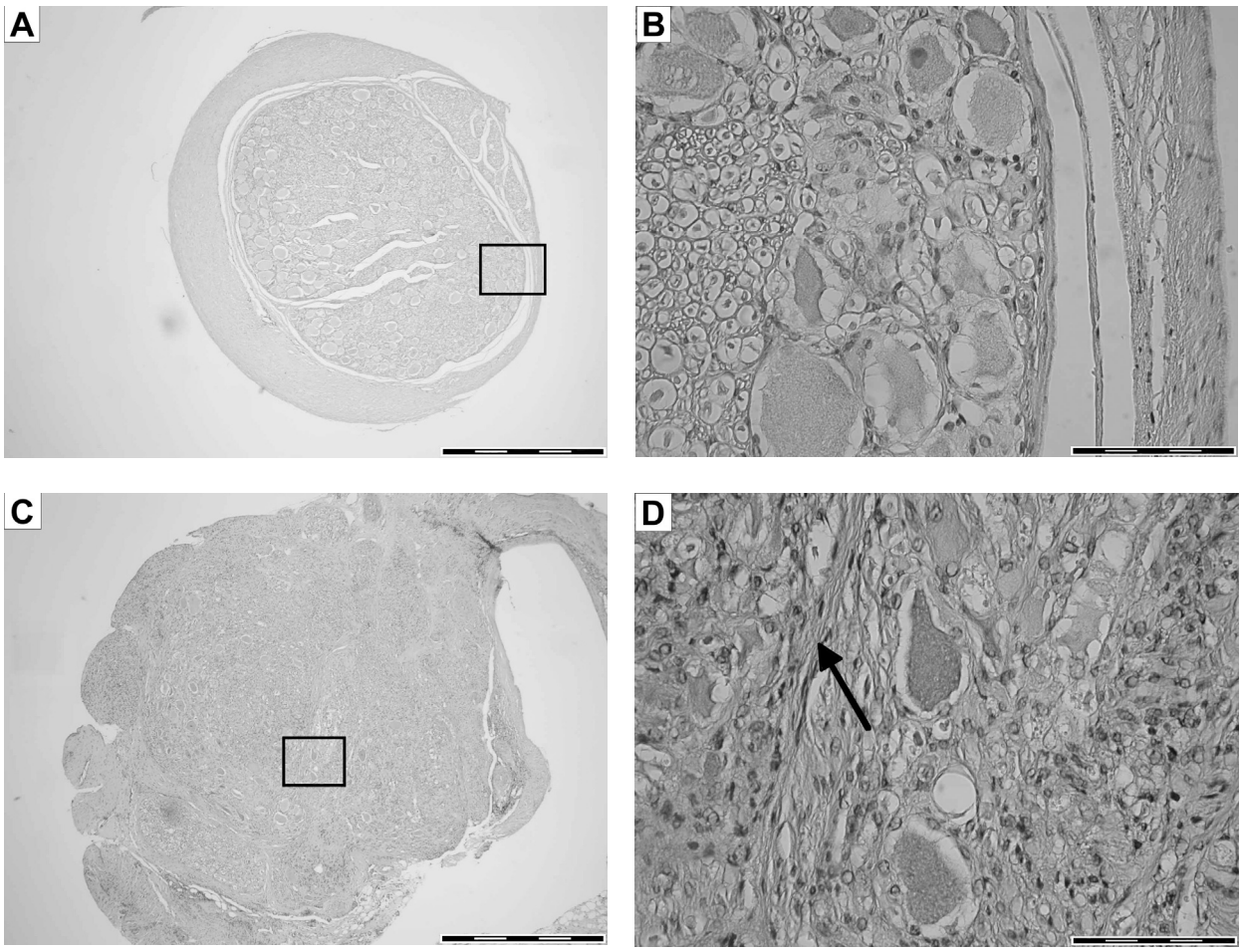

Fig. 9: Transverse sections of nerve (pig 6) with damage mainly at the cuff ends. (A) Low-power view (bar = 1 mm) of a section near the middle of the cuff. Around the cuff location the foreign body response with inflammatory cells inside the nerve. (B) Higher-power view (bar = 0.1 mm) of the area indicated in (A), showing a small inflammatory response in the periphery of the nerve. On the right side, a part of the connective tissue encapsulation can be seen. (C) Low-power view (bar = 1 mm) of a section distal to the cuff but close to the distal cuff end, showing severe histological changes. The void left by the cuff can be seen on the right side. (D) Higher-power view (bar = 0.1 mm) of the area indicated in (C), showing the details of the histological changes in the damaged nerve: degeneration of most of the myelinated fibers, large amount of inflammatory cells, and thickening of the connective tissue separating fascicles (arrow).

Figure 9 shows an example of nerve sections where the histological changes described above can be seen. A reduced neural response of the particular animal (pig 6) to mechanical stimulation of the dermatome, recorded on the day of explantation (42 days after implantation) already suggested nerve damage. Although only a chronic inflammatory response with relative little nerve damage was seen in sections made at the cuff level (Figure 9, a and b), sections at the cuff ends showed large histological changes (Figure 9, c and d).

4 Discussion

In this study we investigated the feasibility of chronically implanting a cuff electrode to record sacral root afferent nerve activity. With a cuff electrode placed on selected extradural sacral nerve roots and connected to a telemetric device, afferent nerve signals were recorded in all 7 animals on the day of implantation. The success of the implants was divers, but one implant was so successful that, in spite of three additional surgeries (re-location, removal and re-implant of a new telemeter), good nerve signals were recorded for an implantation period of more then one year.

An infection in the short-term postoperative period only developed in the animal with a bi-lateral implant. The risk of infection or nerve damage in this animal was expected to be higher than in the other implanted animals because of the increased surgery time, more handling of the nerves, and the amount of implanted foreign materials was twice as much. Histological examination of the nerve section taken at the level of cuff implantation did not show any signs of an infection, and therefore one of the telemeters was suspected as the source of the infection. The telemeter implanted on the left side had been implanted in the first pig, and could have been not properly cleaned. To prevent further infections, used telemeters were later stored in a formaldehyde buffer until preparation for implantation in another animal. No infection occurred afterwards.

The telemeter punctured the skin after a certain time in pig 2 and 3. In pig 2, the infection and edema caused the animal to scratch open the skin. In pig 3, there were no visible edema or skin marks from repetitive scratching, so the puncture probably resulted from a natural process where the body gradually expels foreign objects. In later implants, the telemeters were therefore placed under the layer of subcutaneous fatty tissue and also to a more ventral position, approximately 15 cm lateral to the pelvis.

4.1 Sensory compound action potentials

Electrical stimulation of the clitoral nerve evoked CAPs with different peak-to-peak amplitudes for different stimulation intensities. At low stimulation intensity, only cutaneous receptors and superficial nerve endings close to the stimulation electrode are stimulated. When increasing the stimulation intensity, the area of excitation is increased, activating more receptors and possibly also reaching other close-by nerve branches. As the CAP recorded by the cuff electrode is the summed response of all activated fibers, its shape and amplitude will change with different distributions of activated nerve fibers. Furthermore, the larger diameter nerve fibers beyond the receptors are stimulated directly when stimulating with larger intensities. The point of spike initiation for these larger diameter fibers also shifts more proximal when increasing the stimulation intensity (Loeb and Peck 1996), causing the noted shift to shorter latencies for earliest components of the CAP.

The largest nerve fibers dominate the recorded responses (Stein et al. 1977; Stein and Pearson 1971) but are also the most sensitive to compression (Mackinnon et al. 1984; Sunderland 1978), making cuff electrode recordings sensitive to nerve damage. Loss of large diameter fibers is therefore expected to decrease the amplitude and increase the latency of evoked action potentials. Decreases in CAP amplitude over time were recorded in all except one pig and histological examination of the sections taken from the location of electrode implantation did show damage to mainly the small and large myelinated fibers. Only the CAP responses recorded in pig 3 did not show any significant changes in the short-term post implant period, and resembled findings of other long-term studies (Sinkjær et al. 1999). The day-to-day variability of the recorded responses was probably influenced by the manual stimulation electrode placement, introducing differences in the exact location on the labium major between experiments. Also the position of the animal during stimulation (lying during anesthetized and standing or sitting during awake experiments) changed the exact skin area that was stimulated, and when stimulating the unrestrained awake animal with the larger currents, the animal generated movements due to induced discomfort. A decrease in recorded amplitude during the first few days after implantation has also been reported to relate to a decrease in electrode impedance (Grill and Mortimer 1994; Malek and Mark 1989; Thomsen 1998). Measurement of electrode impedance at each follow-up experiment was however not possible in our set-up because the cuff electrode was permanently connected to the telemeter.

The onset latency of CAP responses increased over time as could be expected when nerve damage occurs and the fast conducting large diameter fibers degenerate. On day 35 of implantation in pig 7, the latency decreased again, but as the recorded amplitude remained very low, probably only a few large fibers had regenerated. The two changes in onset latency in pig 3 coincided however with the use of anesthesia. General anesthetics affect, in addition to the central nervous system, also the thermoregulatory mechanisms (Flecknell 1987). The use of anesthetics, combined with a predisposition of pigs to hypothermia (Basrur et al. 1988), may therefore have been of influence on the difference (0.45 ms in pig 3) found in latencies calculated from the response to supra-maximal electrical stimulation in the awake and anesthetized experiments. The absolute decrease in nerve conduction velocity (CV) due to decrease in body temperature can vary 1.1 to 2.4 m/s/°C (Denys 1991). Electrical stimulation was in general performed at the end of the experiments, after minimal 2-3 hours of general anesthesia (when used). During this time, skin and core body temperature could have decreased sufficiently to cause a significant decrease in nerve conduction (Schwartz 2002).

4.2 Cutaneous nerve signals

Cutaneous receptors are innervated by the largest diameter afferent nerve fibers (Aα and Aβ). The evoked mass neural activity from cutaneous receptors and recorded with whole nerve cuff electrodes is related to the force of the mechanical stimulation, but the receptors are more sensitive to change of force rather than the absolute force (Haugland and Hoffer 1994). Mechanical stimulation of the dermatome by tapping was therefore chosen as methods to induce neural activity because the sensory system is activated in a natural way, and the evoked neural response was expected to be the largest in amplitude when recording from the sacral nerve roots. Manual application of the skin taps does increase the variability of the recorded nerve signal but, after having established a fixed pattern, it could remain relatively constant (see Figure 5, second half of implantation period in upper right graph).

The course of the SNR of the cutaneous nerve signals (Figure 5) was consistent with the CAP response in all pigs (see Figures 2 and 5). In pig 3, it was slightly influenced by the increased level of background activity during the conscious experiments. The background noise level is also influenced by the telemetric system because a poor radio link, e.g. due to increased distance between telemeter and receiver (Donaldson et al. 2003), or electro-magnetic interference from other electronic equipment can induce additional noise.

The median frequency of ENG signals has been positively correlated to the conduction velocity of the fibers recorded from in experimental (Thomsen 1998) and theoretical studies (Struijk 1997), and a decrease can therefore be associated with a reduction in large fibers after neural damage. Assuming the signal recorded as background activity consisted of mainly white noise and the bandpass filtering applied (800 Hz - 4 kHz), the expected median frequency would be around 2400 Hz. The median frequency was however below 2300 Hz in all pigs. This difference is mainly explained by the frequency characteristics of the telemeter system: the overall gain of the telemetric system is not constant, but a maximum in the noise spectrum for frequencies between 1 and 2 kHz (Donaldson et al. 2003). Other reasons for a lower median frequency of the background activity could be the presence of some background neural activity or EMG contamination, inducing more signal power at the lower frequencies, and the special filtering characteristics of the cuff electrode (Struijk 1997). In pig 7, the SNR of recorded nerve responses was significantly lower on day 35 than on the day of implantation. The median frequency was not decreased, but was slightly closer to the median frequency of the background activity. This could have been because the responses were so low that they were not that different anymore than the background activity.

4.3 Histology

The length of the extradural sacral nerve roots in the pigs was limited. There was in general only about one cm or less of free nerve left on either side after placing the cuff electrodes. Short nerve lengths make implantation of the cuff around the nerve difficult, and the handling causes easily nerve damage. Bending of the nerve at the cuff ends can lead to compression neuropathy (Hoffer 1990). To avoid this, and to accommodate the dorsal root ganglion seen in three of the pigs, the diameter of the cuff electrode used was often larger than indicated by the diameter of the nerve. However, until this space between nerve and cuff is filled with connective tissue, mechanical stress on the lead cables will enable bending movements of the cuff electrode and cause compression neuropathy, while the small loop of the lead wires to relief stress is trapped in fibrous tissue after approximately a week, leaving virtually no stress relief. It is therefore not unexpected that most of the observed histological changes occurred at the level of the proximal and distal cuff end. Care should however be taken when relating the histological findings to the electrophysiology, as nerve sections from 3 of the 5 animals were taken several weeks after the last signals were recorded.

The incidence of mechanical nerve trauma may be reduced by using a different cuff electrode design such as the spiral cuff electrode (Naples et al. 1988), reducing the cuff length by recording bipolarly (approx. 50%), or by intradural implantation. The dorsal root is smaller in diameter and possibly longer, there is no ganglion, and no signals from efferent fibers interfere during conscious recordings. Intra-dural electrode placement may offer a good perspective for implantation in humans, where the spinal canal below the level of the L1 vertebrae contains only nerve roots, offering more space and a larger nerve length for a safer cuff electrode application.

5 Conclusion

Results from this study demonstrate that chronic implantation of cuff electrodes for recording of afferent nerve signals from the extradural sacral root is feasible. Furthermore, if nerve damage is inflicted at implantation or in the first few days afterwards, this will be clearly visible in the recorded nerve signals. The time interval between the follow-up experiments was not short enough to conduct a detailed electrophysiological study, and it was therefore difficult to determine exactly when changes occurred. However, amplitude and latency of the compound action potentials and the median frequency, have shown to be useful indicators of possible nerve damage. Histological results could only be related to electrophysiological results in two animals because only in these animals nerve responses were present on the day of explantation. Results may improve when a larger nerve length is available for electrode application, which may be offered by intradural implantation.

Acknowledgements

This study was supported by the Danish National Research Foundation and the Danish Technical Research Council. The authors would like to thank dr. med. Karsten Nielsen M.D. from the Department of Pathology at Aalborg Hospital for preparation of the histological sections, and Morten Haugland Ph.D. for assistance with the cuff electrode implantation during the initial part of the study.

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Chapter 3

Electroneurographic signals from sacral roots in pigs using chronically implanted cuff electrodes.

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Abstract

Under certain neurological conditions, such as spinal cord injury (SCI), the storage function of the urinary bladder can become disrupted by the development of involuntary bladder contractions, which may lead to incontinence, low bladder capacity and damage to the upper urinary tract. These involuntary contractions can be interrupted using electrical stimulation to activate appropriate inhibitory reflex pathways. For this to be feasible as a treatment option, a safe and reliable method to monitor bladder activity on a long-term basis is needed. The objective of this study was to investigate whether bladder activity can be monitored based on the electrical activity recorded from the sacral nerve root using chronically implanted nerve cuff electrodes.

Cuff electrodes were implanted in seven female mini pigs on the extradural sacral root S1 (n=1), S2 (n=4, one bilateral implant), and S3 (n=3) and connected to a telemetric device. The nerve activity was monitored on follow up experiments during bladder filling, rectal distension and mechanical stimulation of the relevant sacral dermatome, and in two pigs also during conscious cystometries.

The duration of implantation ranged from 19 to 383 days. At the first follow-up experiment between 7 and 19 days after implantation, nerve responses to mechanical stimulation of the dermatome were recorded in 5 pigs, responses to rectal distension in 4 pigs, and responses to bladder filling in 3 pigs. The nerve recordings during conscious cystometries showed a characteristic pattern of nerve activity mainly related to movement of the hind limbs and rhythmic perineal and urethral contractions during and after voiding.

The results demonstrate that sacral root nerve responses to afferent stimulation of different pelvic organs can be recorded using chronically implanted cuff electrodes. However, the complex pattern of neural activity recorded during the conscious cystometries demonstrates that further improvements in recording selectivity are needed for a feasible clinical application of monitoring bladder activity.

1 Introduction

Normal filling of the urinary bladder occurs with little or no change in pressure and involuntary contractions are absent. However, under certain neurological conditions, such as spinal cord injury (SCI), the filling phase is characterized by involuntary contractions of the detrusor muscle. This condition is referred to as neurogenic detrusor overactivity (NDO) (Abrams et al. 2002), which often causes incontinence and in severe cases also damage to the upper urinary tract. Current pharmacological and surgical treatments have however variable success rates or have side effects (Baigrie et al. 1988; Chancellor and De Groat 1999; Hohenfellner et al. 2001; Leng et al. 1999; Schurch et al. 2000).

An alternative treatment option relies on activating existing inhibitory reflexes by electrical stimulation of afferent nerve fibers carried by anorectal branches of the pelvic nerves and the dorsal penile or clitoral branches of the pudendal nerve (Fall and Lindstrom 1991). Stimulation does however not need to be continuous. Conditional stimulation, i.e. stimulating only when a detrusor contraction occurs, has been shown effective in inhibiting reflex contractions evoked by rapid saline infusions (Shah et al. 1998), cystometries (Dalmose et al. 2003; Kirkham et al. 2001), and during natural bladder filling (Hansen et al. 2005). Applying stimulation conditionally will also reduce energy consumption in battery-powered systems and minimize habituation to repetitive activation of the inhibitory reflexes.

For conditional stimulation to be feasible as a treatment option, a safe and reliable method to monitor intravesical pressure on a long-term basis is needed. With the advent of methods for long-term electrical interfacing with nerves, recording from the natural sensors in the human body has become a realistic alternative (Sinkjær et al. 1999). Sensory nerve signals related to mechanical bladder activity could be recorded and used to detect and inhibit involuntary bladder contractions (Jezernik 2000). Earlier studies demonstrated an increase in single nerve fiber activity with increased tension in the bladder wall and bladder pressure (Habler et al. 1993; Winter 1971). More recently, electroneurographic (ENG) signals reflecting increases in bladder pressure have been recorded in acute experiments using cuff electrodes placed on the pelvic nerves and sacral nerve roots in pigs (Jezernik et al. 2000), cats (Jezernik et al. 2001a) and human (Kurstjens et al. 2005).

These previous acute recordings were performed in response to mechanical stimulation of only one single pelvic organ (bladder, rectum, or skin) at a time and during general anesthesia. However, as the sacral roots do not innervate the bladder only, it can be expected that under normal conditions the recorded whole nerve signal is the summed neural activity of different efferent and afferent origin. The recorded nerve signal will in that case only resemble bladder activity if the contribution from the bladder afferents is considerably larger than all other nerve activity. In addition, many anesthetics used in acute experiments have an adverse affect on micturition physiology (Matsuura and Downie 2000; Rudy et al. 1991), which may lead to a neural response different than normal, and possible responses can also be detected easier within a lower level of neural background activity when the central nervous system is suppressed. These drawbacks may however be avoided in a chronic setting.

We therefore implanted cuff electrodes on extradural sacral nerve roots and investigated if afferent nerve signals originating from different pelvic organs can be recorded chronically. The present study focused on characterizing the recorded neural signals, whereas the effects of the chronic implantation on the neural interface were investigated in more detail in another manuscript (Kurstjens et al. 2008).

2 Material and Methods

2.1 Implantation procedure

The sacral roots in 7 female Göttingen mini-pigs (weight 29 to 45 kg) were exposed by a dorsal laminectomy under general anesthesia induced by intra-muscular Midazolam (0.1 ml/kg) and Ketaminol (0.2 ml/kg), and maintained with 1.5% Isoflurane. Tri-polar cuff electrodes (inner diameter 1.8 to 3.0 mm, length 15 mm, platinum ring contacts 1 mm wide) were implanted on the extradural sacral nerve root that showed the largest increase in bladder pressure in response to electrical stimulation (20Hz, 200 μs pulse duration, 0.5-1 mA in amplitude) with a hook electrode. To allow chronic monitoring of the nerve activity, the cuff electrode was connected to a telemetric device (Donaldson et al. 2003) that was placed in a subcutaneous pocked just lateral to the spine and approximately 5 cm cranial to the pelvis.

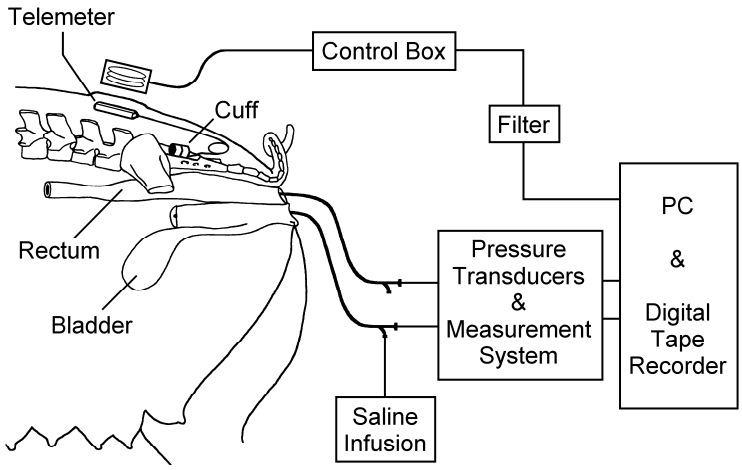

Fig. 1: Schematic diagram of the experimental set-up.

2.2 Experimental protocol

Before each follow-up experiment, a urine dipstick test (Multistix®, Bayer Co., USA) was performed to test the animals for cystitis. Follow up experiments were initially performed under general anesthesia, but conscious recordings were also performed later on in the implantation period of three pigs. The experimental set-up is shown in Figure 1. In order to investigate the various afferent sources contributing to the sacral root recordings, mechanical stimulation of the following organs was performed:

Dermatome: The relevant sacral dermatome was stimulated by manual rubbing.

Rectum: A rectal balloon consisting of a latex glove mounted on a standard 16-F catheter (Rüsh, Kernen, Germany) was used to distend the rectum. It was filled by one or two 100 ml injections of saline. This was then followed by repetitive removing and re-injecting 50 ml.

Bladder: The empty bladder was filled with 50 or 100 ml saline injections until the bladder volume reached 300-400 ml. Conscious cystometries were performed in two pigs: in one pig on 10 different days between 98 and 363 days after implantation, and in the other pig on 3 different days between 21 and 91 days after implantation. The conscious animals were placed in a restriction cage and a roller pump was used for continues saline infusion (rate: 60 ml/min). Infusion was stopped at the onset of voiding.

Control measurements were performed in two pigs at the end of the implantation period. Sensory compound action potentials (CAP) were elicited by electrical stimulation with a bipolar electrode (HUSH-bar, Dantec-Medtronic, Skovlunde, Denmark), placed on the skin over the dorsal clitoral nerve (lateral to the Labium Major), before and after the pudendal nerve (PN) was cut. Stimulation was performed using rectangular pulses, 6 Hz, 200 μs pulse duration, and 1-32 mA amplitude. In one of these two pigs, the bladder was pressed strongly by applying a pressure manually from outside and inside the abdominal cavity, both before and after the PN was cut.

2.3 Urodynamic monitoring

Rectal and intra-vesical pressures were recorded via fluid filled lines connected to pressure transducers (PX-600F TruWave DPT, Edwards Lifesciences, USA) and a recording system (CM4008, CardioMed, Norway). The rectal pressure was measured using a 10-F balloon catheter (Rüsh, Kernen, Germany). For bladder filling and measurement of the bladder and urethral pressure, a separation catheter was made: a size 12 - 16 F three lumen catheter (Rüsh, Kernen, Germany) with a size 4-F catheter (Vygon, Ecouen, France) attached (Dalmose et al. 2002). The catheter balloon was filled with 15ml saline and the catheter was gently retracted into the bladder neck to create an outlet obstruction. The intravesical pressure during obstructed voiding was expected to rise higher than during unobstructed voiding and thereby evoking a larger amount of afferent nerve activity. The balloon was deflated 2-5 minutes after the onset of voiding contractions to allow bladder emptying.

2.4 Data processing and analysis

The ENG signal received from the telemeter was band-pass filtered (400 Hz - 4 kHz) before being sampled at 20 kHz, full-wave rectified and bin-integrated (RBI, 50 ms bin duration), and stored on a PC together with bladder and rectal pressure. The unprocessed ENG and pressure signals were also stored on a digital tape recorder (RD-135T, TEAC, Japan) to allow later offline analysis.

Evoked neural responses were quantified by the signal-to-noise ratio (SNR) as the ratio between the peak RBI ENG and the background activity (BGN) when no stimulation was applied (corrected for the level of BGN, and averaged over 0.5 s). The relation between changes in bladder or rectal pressure due to manipulations and evoked neural response was expressed by the cross-correlation coefficient (r).

A t-test was used to compare the levels of recorded ENG before and during voiding. A one-way ANOVA analysis was used to test the influence of the catheter balloon during voiding on recorded ENG.

3 Results

In the 7 pigs that were implanted, cuff electrodes were placed on the S1 (n=1), S2 (n=4, one pig bilaterally implanted), and S3 (n=3) extradural sacral nerve roots. The duration of implantation ranged from 19 to 383 days, after which the animals were sacrificed for reasons varying from electrode failure (2 pigs), skin puncture (1 pig), infection (1 pig), suspected brain damage after anesthesia (1 pig), to nerve damage (2 pig). No signs of urinary tract infections were encountered at any follow up experiment.

3.1 Nerve signals recorded during mechanical stimulation of the dermatome

Nerve signals were recorded in all 7 animals when mechanical stimulating the relevant dermatome on the day of implantation. Figure 2 shows an example of the neural response recorded following contact with the skin. The fast adaptation of the cutaneous mechanoreceptors in combination with the repetitive rubbing caused variations in the amplitude of the recorded raw ENG signal, of which only peak activity exceeded the level of noise and background activity (Figure 2, upper trace). Application of the RBI procedure improved the SNR and demonstrated also the presence of ENG signals with very small amplitudes (Figure 2, lower trace).

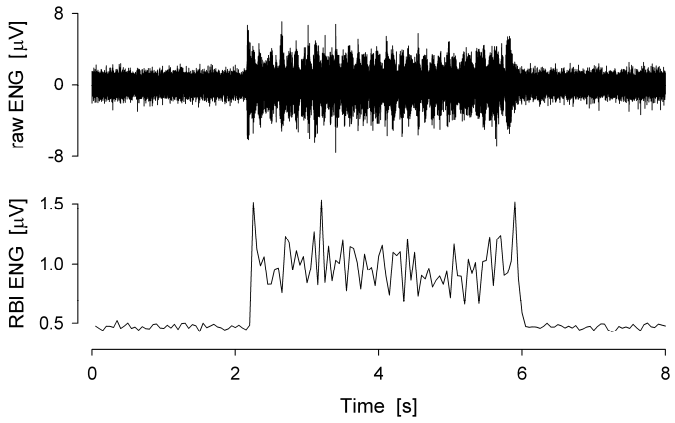

Fig. 2: Nerve activity recorded from the left sacral root S2 when rubbing the dermatome of pig 6 by hand on the day of implantation. The upper trace shows the raw nerve signal and the lower trace shows the signal after band-pass filtering, rectification and bin integration.

The average SNR of the ENG response recorded on the day of implantation ranged from 0.29±0.23 to 1.60±0.67 for the different pigs. At the first follow-up experiment, between 7 and 19 days after implantation, nerve responses were recorded in 5 out of 7 pigs, however with a significantly lower SNR of between 0.14±0.14 and 1.34±0.37 compared to immediate after implantation ($p=0.016$, paired t-test). After 35 days of implantation, nerve responses were recorded in 2 pigs with a SNR of 0.33±0.13 (pig 6) and 0.16±0.08 (pig 7) respectively, and after 42 days only in pig 7 (SNR = 0.30±0.11). In one pig (pig 3) the telemeter started to malfunction after 28 days, but when it was removed on day 59 and a new device was implanted on day 84, nerve responses with a SNR of 1.96±0.51 were recorded. This implant remained then stable with SNR's varying between 1.61±0.41 and 3.24±0.63 recorded on 10 different experiment days during the rest of the implantation period. When the animal was sacrificed after 383 days, nerve responses were recorded with a SNR of 2.80±0.62.

3.2 Nerve signals recorded during rectal distension

Increase in neural activity during rectal distensions was recorded in 4 out of 5 pigs at the first follow-up experiment. No increase was recorded in the pig with the cuff electrode implanted on the S3 root, while in pig 3 (cuff on S1) an increase in neural activity was also recorded during rectal distensions on the day of termination. The generated rectal pressure profiles and amount of evoked neural activity depended on the infusion rate, which could be classified as slow (< 6 cm H2O/s) or fast (> 8 cm H2O/s), as can be seen in Table 1.

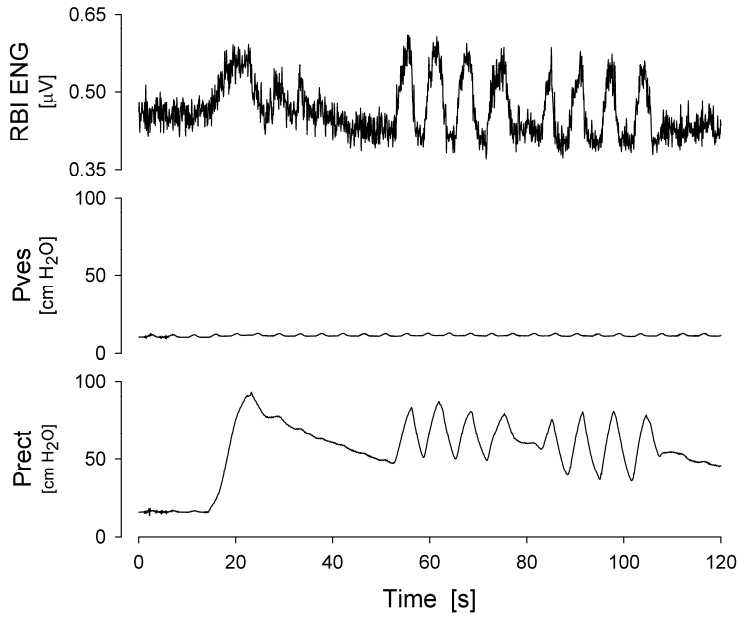

Fig. 3: Nerve responses to rectal distension recorded from the right sacral root S2 in pig 2, 14 days after implantation. Large phasic nerve responses to increases in rectal pressure were recorded in during rapid saline infusions into the rectal balloon, first a 100 ml infusion followed by a series of retraction and rapid re-infusion of 50 ml.

An example of the neural activity and pressure signals recorded during a series of rectal distensions in pig 2 are shown in Figure 3. The rectal balloon contained already 100 ml when at t = 15 s an additional 100 ml was infused. Approximately 50 ml was retracted back into the bolus around t = 40 s, and then used for a series of fast infusions and retractions. The pressure in the rectum increased during the saline infusions into the balloon, but decreases gradually when infusions were stopped and rapidly when fluid was retracted. Increases in pressure were immediately followed by a phasic ENG response. The tonic ENG response to a sustained high pressure in the rectum was usually low, and a decrease below the level of background activity was often observed during fast retractions.

The SNR and cross correlation coefficients obtained for ENG signals recorded during the rectal distensions in the anesthetized animals are summarized in Table 1. There were no significant differences in the correlation coefficients between ENG and rectal pressure when infusing the initial 100 ml in an empty rectal balloon with fast or slow rates for signals recorded from S1 and S2. However, the SNR of the ENG signals was significantly higher during additional fast than slow infusions for signals from both S1 ($p = 0.007$) and S2 ($p = 0.017$). When retracting and infusing an additional 50 ml, no significant differences were found for the correlation coefficient and SNR between slow and fast infusion rates for ENG signals recorded from S1. For ENG signals recorded from S2 however, the correlation coefficients and SNR obtained during fast distensions were significantly larger ($p < 0.01$) than during slow distensions. In the pig that was implanted bilaterally on the S2 roots, no significant differences were found between roots, but the SNR was higher than in the other two pigs implanted on S2 ($p < 0.035$, SNK). The SNR and cross-correlation coefficients of ENG responses to changes in rectal pressure in the pig with the cuff electrode implanted on S1 were significantly higher ($p<0.01$) compared to the three pigs that were implanted on S2.

A		S1 (n=1)					
		n	Pmin [cm H_2O]	Pmax [cm H_2O]	Rate [cm H_2O/s]	r [-]	SNR [-]
Initial Infusion	S	5	7.5± 7.0	66.5±10.9	3.5±1.5	0.69±0.10	0.32±0.07
	F	8	11.5± 4.0	88.8±28.8	17.1±9.3	0.81±0.12	0.57±0.15
Additional Infusion	S	5	31.4± 3.6	83.0± 8.7	4.62±2.6	0.86±0.07	0.52±0.12
	F	27	36.3±17.9	102.8±22.6	22.7±9.5	0.81±0.11	0.64±0.13

B		S2 (n=3)					
		n	Pmin [cm H_2O]	Pmax [cm H_2O]	Rate [cm H_2O/s]	r [-]	SNR [-]
Initial Infusion	S	8	13.5± 7.3	44.4± 8.6	3.6±2.3	0.41±0.15	0.10±0.03
	F	6	11.1± 4.3	66.7±23.7	8.4±0.4	0.52±0.14	0.18±0.07
Additional Infusion	S	3	23.9± 5.8	59.1± 8.4	5.4±1.6	0.43±0.14	0.07±0.02
	F	15	38.5±14.8	74.3± 9.2	10.8±2.1	0.63±0.13	0.22±0.12

Table 1. Grand means of rectal pressures (Pmin and Pmax), infusion rate, SNR and coefficients of cross-correlation (r) between ENG and rectal pressure during fast (F) and slow (S) rectal distensions, and for cuff electrodes implanted on **(A)** S1, and **(B)** S2 sacral nerve roots.

Rectal distensions were also performed in one conscious animal (pig 3) on different days between 119 and 363 days after implant. Movement of the rectal balloon often triggered reflex defecation, causing the balloon to be expelled. Although large ENG signals (SNR ≤ 2.20) were recorded during these defecations, they were however not related to the movements in the rectum but of the hind limb. Nevertheless, in 16 out of 25 undisturbed distensions, a small increase in ENG could be recorded in response to slow infusions (SNR = 0.16±0.09, r = 0.51±0.19, dPrect = 16.4±4.6 cmH2O, rate = 3.3±1.7 cmH2O/s, n=8) and fast infusions (SNR = 0.11±0.12, r = 0.43±0.20, dPrect = 73.9±4.0 cmH2O, rate = 32.0±5.1 cmH2O/s, n = 5).

3.3 Nerve signals recorded during bladder filling

An increase in nerve activity was present in 3 out of 4 pigs (S1, S2, S3, respectively one pig each) where an increase in bladder pressure of 24 - 55 cmH2O was obtained with bladder filling on the first follow-up. The ENG responses were small (SNR = 0.03-0.13). The correlation between ENG responses and Pves was low (r = 0.04 - 0.45) because of the low amplitude and phasic nature of the ENG responses.

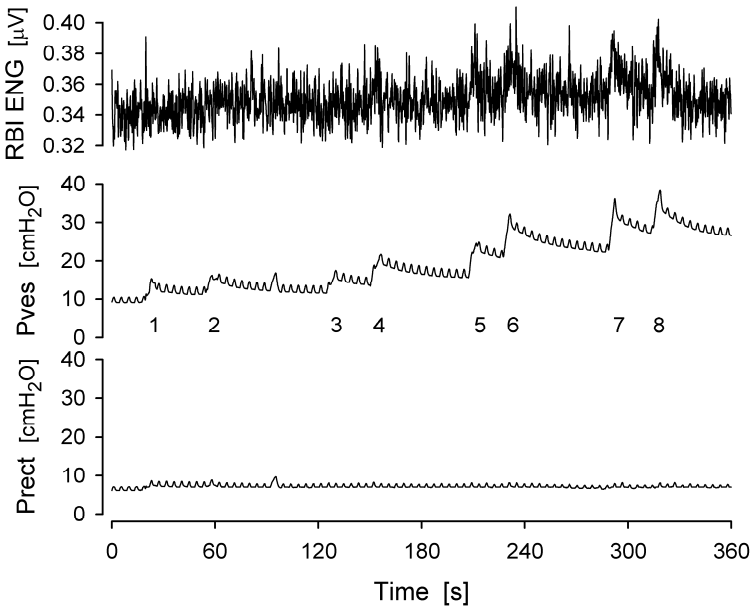

Fig. 4: Nerve responses to bladder filling recorded from the left sacral root S2 in pig 2, 14 days after implantation. Eight consecutive saline boluses of 50ml each (indicated by the numbers 1-8) were infused into to bladder.

A recording during manual infusion in the pig 2 (bilateral implant) is shown in fig. 4. The intravesical pressure increased with each saline infusion (50ml). Small ENG responses (SNR = 0.09 - 0.13) were present during the fourth to eighth infusion, and the correlation between Pves and ENG increased from 0.12 to 0.43 with these subsequent infusions. Although the increases in Pves were similar when repeating the filling and recording from the contra lateral sacral root, nerve responses were only present during the last two out of eight infusions (SNR = 0.08 and 0.13, with r = 0.36 and 0.37 respectively).

3.4 Nerve signals recorded during conscious cystometries

During conscious cystometries (n = 30) that were performed in pig 3, 37 voidings were recorded, and 5 voidings were recorded during cystometries (n = 5) in pig 7. The infused volume ranged from 20 to 1000 ml (mean: 436 ml) for pig 3 and from 450 to 1000 ml (mean: 727 ml) in pig 7, and the voided volume was respectively 83 to 910 ml (mean: 405 ml) for pig 3 and 410 to 910 ml (mean: 646 ml) for pig 7.

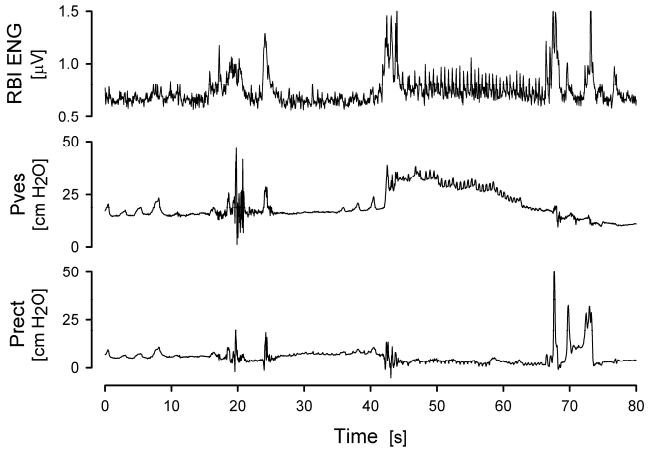

Fig. 5: Nerve responses recorded from the left sacral root S1 during a conscious cystometry on day 126 of implantation in pig 3. Large increase in nerve activity were recorded during movements of the pig following the onset of infusion, see e.g. around t = 20, 25, 43, and 68 s, Movement artefacts are also reflected in the both pressure signals. Note also the increases in ENG during activity in the rectum (t = 70 s).

The ENG and pressure signals recorded during the cystometries in pig 3 followed a characteristic pattern. A typical example is shown in Fig. 5. Voiding occurred approximately 30 s after filling started. Sensations by the animal of the onset of infusion often lead to hind limb and tail movements, of which related neural activity was recorded as large increases in the ENG signal (SNR up to 5), as can be seen at time around 20s. When the desire to void occurred, large increases in ENG again reflected the hind limb and tail movements initiated in preparation of the voiding (squatting, time = 40 – 45s). During the voiding, a small increase in baseline ENG can be seen. Such small increases in baseline ENG activity were present during 31 out of 37 voidings ($p < 0.03$). Voidings occurred in general not by continues urine flow, but rather in a squirting manner. Because the corresponding rhythmic activity was more often present in the recorded ENG than in the bladder pressure during voiding and the frequency of this rhythmic activity was too high to be associated with mechanical activity of the bladder, the pressure in the urethra was also recorded during later cystometries (n = 20).

Figure 6 shows the ENG and pressure signals recorded during a typical cystometry when also urethral pressure was measured. The same characteristic pattern as shown in Fig. 4 can be recognized. In addition, single bursts of ENG activity observed during the filling phase without simultaneous activity in the bladder or rectum, could be correlated with activity in the urethra. These short bursts of ENG coincided with sudden urethral relaxations (time = 1-3 minutes, Fig. 5) and generally occurred more often near the end of the filling phase. One such urethral relaxation is shown in detail in Fig. 7A.

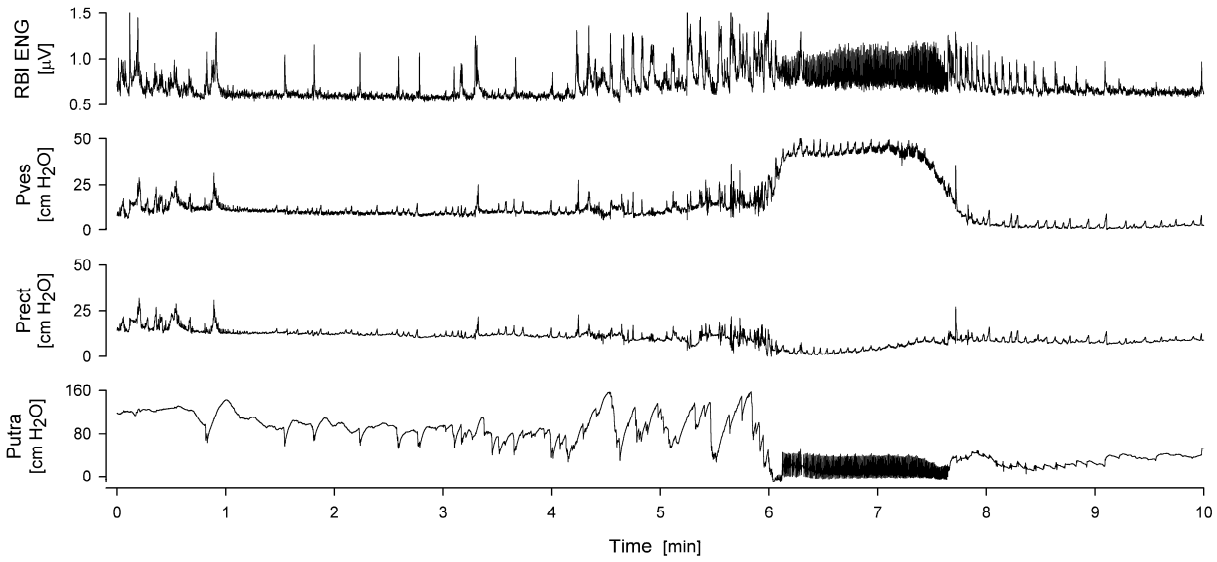

Fig. 6: Nerve responses recorded during a conscious cystometry with bladder outlet obstruction, 154 days after implantation in pig 3. Many events in the recorded nerve activity were related to changes in urethral pressure, and not bladder or rectal pressure.

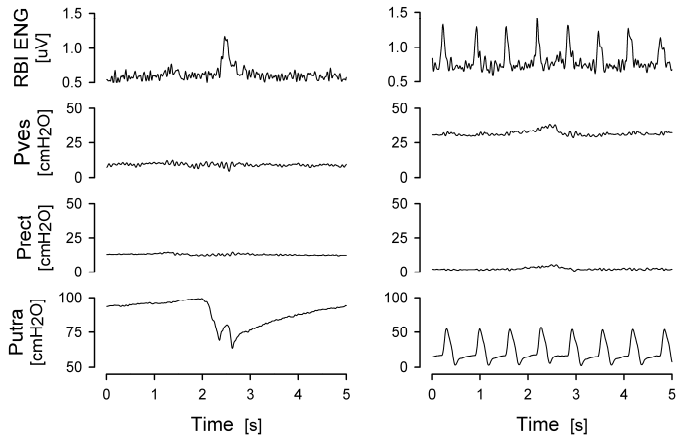

Fig. 7: Details of Figure 6 but using a smaller time constant for the bin integration, showing clear correlation between recorded ENG and pressure changes in the urethra. On the left, sudden drops in pressure in the urethra during the filling phase were immediately followed by a burst of nerve activity to restore the pressure. On the right, bursts of efferent nerve activity initiating rhythmic contractions in the urethra during a bladder contraction with obstructed outlet.

Although the intravesical pressure during cystometries, where the transurethral catheter balloon was inflated (obstruction of the bladder outlet), did reach higher values (Pves = 54±10 cmH2O, n = 26 voidings) than during non-obstructed voiding (Pves = 37±13 cmH2O, n = 10 voidings), there was no significant difference in increase of recorded baseline ENG ($p = 0.604$). The high intravesical pressure was in general sustained until the balloon was deflated. Phasic urethral contractions and relaxations were clearly reflected in the recorded ENG and urethral

pressure during the sustained higher bladder pressures (Fig. 7B), and urine leakage occurred often drop-by-drop. When using a shorter time window for bin integration (Tbin = 10 ms), the cross correlation function between this phasic activity in the ENG signal and urethral pressure showed a lag of 90±22 ms (n = 16 voidings) for the urethral pressure. The outlet obstruction also evoked abdominal straining, generating an increase in intravesical and rectal pressure (range: 150 – 200 cmH2O) and large ENG signals (SNR up to 3.5).

Large increases in ENG signal were recorded again when tail and hind limb movements indicated the end of the voiding. This was generally followed by rhythmic perineal contractions, which were typically reflected in the recorded ENG and urethral pressure (Fig. 6, time = 8-9 min.). The pressure variations during these perineal contractions were often similar to the pressure variations of the urethral relaxations during filling, and lagging the peaks in ENG by 120±33 ms (n = 42).

As the absence of cutaneous ENG responses on day 21 after implantation in pig 7 suggested nerve damage, conscious cystometries were performed to test bladder function. All cystometries resulted in normal voidings and the recorded bladder, rectal and urethral pressure profile included characteristics similar to those recorded in pig 3. No ENG responses were recorded during the cystometry on day 21, but on day 35 there was a small increase in baseline ENG present during bladder contractions (n= 2) whereas no ENG responses were recorded during urethral or perineal contractions and also during hind limb movements the ENG signal was greatly reduced. No ENG responses were recorded during conscious cystometries (n= 2) on day 91 after implantation.

3.5 Control experiment

In two animals (pigs 3 and 6) a cutaneous ENG signal was present at the end of the implantation period, and a control experiment was performed before the animals were sacrificed. Under general anesthesia, the ipsilateral pudendal nerve (PN) was cut during mechanical stimulation of the dermatome. No cutaneous ENG signals were recorded after the PN was cut in pig 3, but in pig 6 the amplitude of the recorded ENG was only reduced by approximately 50%. Nerve transsections were verified by comparing sensory compound action potentials (CAP) elicited by transcutaneous electrical stimulation of the clitoral nerve before and after the PN was cut. Figure 8 shows a CAP response recorded in pig 3. No CAP responses were recorded in both pigs after the PN was cut.

ENG signals recorded during rectal distensions in pig 3 were significantly reduced in amplitude after cutting the PN (SNR = 0.28±0.05, n = 7) compared to before (SNR = 0.57±0.20, n = 9), but still correlated well with the changes applied in rectal pressure (before: r = 0.82±0.08, n = 9, and after: r = 0.73±0.09, n = 7). The level of background ENG activity in recordings made after cutting the PN was significantly lower in both pigs 3 and 7 than before it was cut ($p < 0.022$).

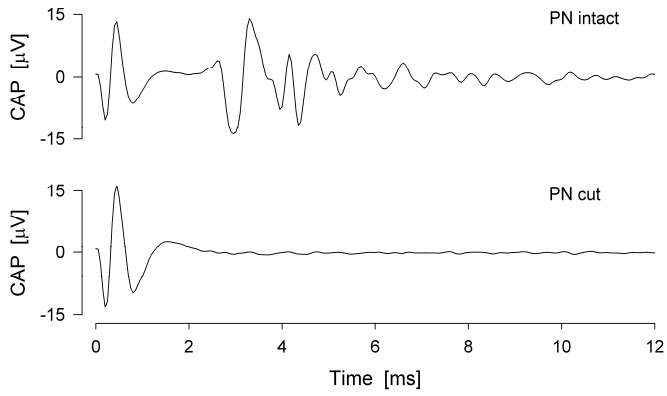

Fig. 8: Averaged CAP response to electrical stimulation (I = 32 mA, N = 38) of the clitoral nerve before (upper trace) and after (lower trace) the pudendal nerve (PN) was cut in pig 3 after 383 days of implantation.

Phasic nerve responses with SNR = 0.32±0.11 and r = 0.59±0.15 (n = 7) were present in pig 3 during manual pressing from outside the abdomen. However, after the PN was cut, nerve responses decreased and the correlation with bladder pressure became negative (SNR = -0.13±0.02, r = -0.60±0.10, n = 8). Also after the PN was cut, intravesical pressures obtained by strongly pressing the bladder by hand from inside the abdominal cavity (Pves = 80±7 cmH2O, n = 11) were larger than those during pressing from the outside (37±15 cmH2O), and ENG responses with SNR = 0.19±0.07 and r = 0.55±0.18 (n = 11) were recorded (Fig. 9).

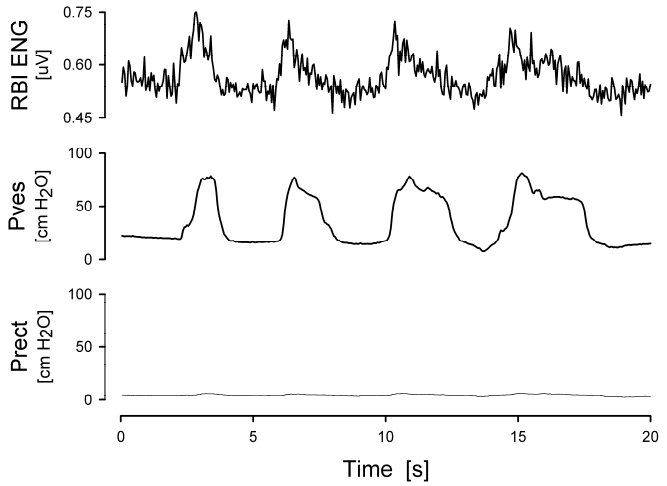

Fig. 9: Nerve activity recorded when the bladder was pressed strongly by hand from inside the abdominal cavity following transsection of the pudendal nerve in pig 3 after 383 days of implantation.

4 Discussion and conclusions

The objective of this study was to record nerve activity from the extradural sacral nerve roots using chronically implanted cuff electrodes. The results demonstrate that this is possible. The results demonstrate however also the many difficulties that occurred. The duration of implantation varied between pigs for different reasons, and only 3 out of 7 implanted pigs showed afferent nerve responses after 30 days. Nevertheless, the implantation in one pig was functional for more than one year and reflex activity of the bladder and rectum was evoked during many conscious follow-up studies, while simultaneously recording the overall nerve activity. To our knowledge this particular implant demonstrates the first recordings of sacral root nerve activity in a conscious animal using chronically implanted cuff electrodes.

The amplitude of nerve responses evoked by mechanical stimulation of the dermatome was always larger than responses recorded during stimulation of the bladder and rectum. The cutaneous mechanoreceptors are innervated by myelinated nerve fibers with a larger diameter than the bladder and rectum (smaller myelinated and unmyelinated fibers). Therefore, since the aggregate nerve activity recorded from a whole nerve depends on the number of active fibers and is dominated by the activity of the largest axons (Hoffer and Kallesøe 2001), the largest amount of recorded afferent nerve activity was from the cutaneous afferents.

Consistent nerve responses were recorded from the S1 and S2 sacral roots during rectal distension at the first follow-up experiment. The nature of the responses was similar to the nerve responses recorded during bladder fillings in an earlier acute study in pigs (Jezernik et al. 2000). The SNR and cross correlation between nerve responses and rectal pressure recorded during fast distensions were larger than during slow distensions. This result indicates that the rectal afferents are more sensitive to phasic stimuli, or that the cuff electrode records mainly from rectal afferents responding to phasic stimuli. Pelvic nerve afferents exhibiting phasic or tonic responses to rectal distension have also been found in cats (Janig and Koltzenburg 1991) and rats (Sengupta and Gebhart 1994). Furthermore, the SNR and cross correlation of the responses recorded from the S1 root were larger than those recorded for S2. This could be because that S1 root carried a larger number of rectal afferents, or because the diameter of the cuff in this particular implant was smaller than of the cuffs used for the S2 implants. Rectal manipulations during conscious distensions often triggered reflex defecation, during which amplitudes of nerve responses were up to 20 times larger than during anesthetized distensions. These large nerve responses were most likely related to efferent and cutaneous afferent nerve and pick-up of nearby muscle activity related to limb and tail movements.

In previous acute studies bladder afferent responses were evoked by infusion of saline into the bladder in pigs (Jezernik et al. 2000), cats (Jezernik et al. 2001a), and human (Kurstjens et al. 2005), and it was assumed that recorded whole sacral root nerve activity originated from the bladder only. In the present study, similar nerve responses were also recorded during bladder fillings at follow-up experiments using long-term implanted cuff electrodes. The nerve activity recorded during the conscious cystometries in pig 3 was however far more complex and hardly resembled changes in bladder pressure. However, it did follow a characteristic pattern and after including measurement of the urethral pressure and observing the behaviour of the pig during the cystometries, most of the recorded nerve responses could be explained. Large nerve responses were recorded a) at the onset of infusion, b) when squatting to prepare to void, and c) immediately after the voiding was finished. This represented the summed activity of afferent and efferent nerve fibers innervating different pelvic viscera, the skin, and lower limb musculature,

all involved in the latter mentioned events. Although recorded from the sacral root, most of this nerve activity originates from fibers supplying the pudendal nerve or nerves innervating the hind limbs, tail and dermatome, and not from the bladder. For example, the peaks in the ENG signal recorded during urethral and perineal contractions were leading correlating changes in urethral and rectal pressure, indicating they were of efferent origin.

The implantation in pig 7 also demonstrated that the majority the recorded sacral root ENG originates from other sources than the bladder. Although the amplitude of recorded nerve activity is known to decrease during the first few days of implantation because of fluid accumulation (Grill and Mortimer 1994), after 21 days there were no ENG responses present in pig 7 and therefore nerve damage had likely occurred. Nevertheless, a small increase in background level was recorded during bladder contractions during the conscious cystometries on day 35. This could have been the response from bladder afferents, as mechanical stress or damage to the nerve most severely effects the larger diameter myelinated nerve fibers (Mackinnon et al. 1984; Sunderland 1978), such as the cutaneous afferents and most efferent fibers. This would considerably reduce the contribution of these fibers to the recorded nerve activity, whilst affecting less the contribution from the small diameter visceral afferents.

During unobstructed voidings the recorded increases in baseline nerve activity were only small. The female pig is known to void with low intravesical pressure compared to humans (Dalmose et al. 2000), it was thought that the pressures generated voluntary were not large enough to activate a sufficient number of bladder afferents to record a clear increase in whole nerve activity. Obstruction of the bladder outlet did increase the intravesical pressure but the increase may not have been sufficient as the increase in recorded baseline nerve activity was not larger than during unobstructed voiding.

The control experiment with pudendal nerve transsection in two pigs demonstrated that most of the nerve activity recorded when pressing the bladder from outside the abdominal cavity (Jezernik et al. 2000) originated from the skin or urethra (catheter movement), and not from the bladder itself. Squeezing the bladder manually inside the abdominal cavity after pudendal nerve transsection in pig 3 demonstrated that afferent nerve activity from the bladder can be recorded from a cuff electrode that has been implanted on the sacral root for more the one year. Detected changes in background nerve activity after nerve transaction should however be interpreted cautiously because the decreases were only small and other factors such as changes in the level of anesthesia for the surgical procedure and possible deterioration of the battery condition in the receiver control box, could have been of influence.

The lack of increases in ENG related to bladder activity during the conscious cystometries demonstrated that further improvements in recording selectivity are needed for a feasible clinical application of recording sacral root afferent nerve activity to monitor bladder activity. Electrode implantation on the intra-dural dorsal sacral root would eliminate most of the efferent contribution, whilst at the same time the amplitude of recorded nerve responses may increase as smaller diameter cuff electrodes can be used. The larger space available in the spinal canal would also offer safer electrode application, as supported by the experience with the FineTech-Brindley Bladder System (Brindley 1994; van Kerrebroeck et al. 1993). Furthermore, because various afferent sources contribute to the sacral roots, greater selectivity towards organ specific afferent fibers is needed. This might be achieved using advanced signal processing techniques (Jezernik et al. 2001b; Jezernik and Grill 2001), as well as innovative developments in electrode design (Rieger et al. 2004; Taylor et al. 2004). Although evaluated only in theory and anesthetized animal models, their application might improve selectivity towards activity

originating from bladder afferent nerve fibers in clinical application considering that the complexity of recorded nerve activity is expected to be far less in patients with a spinal cord injury where motor efferent nerve activity is nearly absent.

Acknowledgements

This study has received financial support from the Danish National Research Foundation and the Danish Technical Research Council.

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Chapter 4

Intra-operative recording of electroneurographic signals from cuff electrodes on extradural sacral roots in spinal cord injured patients

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The Journal of Urology, vol. 174, pp 1482-1487, 2005.
DOI: 10.1097/01.ju.0000173005.70269.9c

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Abstract
Purpose: A safe and reliable method for monitoring intravesical pressure on a long-term basis is needed for conditional electrical stimulation to be feasible as a treatment option for neurogenic detrusor overactivity in patients with a spinal cord injury (SCI). Therefore, we investigate the possibility of recording afferent nerve activity related to mechanical activity of the bladder and other pelvic organs from the extradural sacral nerve root in human.
Materials and Methods: Nerve cuff electrodes were temporary placed on the extradural S3 sacral root in 6 SCI-patients who underwent implantation of an extradural FineTech-Brindley Bladder System (Finetech Medical Lt. Welwyn Garden City, United Kingdom). The dorsal penile/clitoral nerve was electrically stimulated to evoke compound action potentials. Electroneurographic (ENG) signals were recorded together with bladder and rectal pressure during mechanical stimulation of the dermatome, rapid bladder filling and rectal distension, and during bladder contraction evoked by electrical stimulation of the contralateral sacral root.
Results: Compound action potentials and electroneurographic responses during stimulation of the dermatome and rectum were present in all 6 patients, and during bladder fillings in 5 of 6 patients. However, recorded responses from the bladder and rectum were small and mainly phasic in nature. Nerve responses following bladder contractions were present in 4 of 5 stimulated patients.
Conclusions: Afferent nerve activity from the dermatome, bladder, and rectum can be recorded using cuff electrodes placed on the extradural S3 sacral root in human but improvements in recording quality and sophisticated signal processing methods are needed for chronic application.

1 Introduction

Electrical stimulation of pudendal nerve afferents can inhibit the micturition reflex (Fall and Lindstrom 1991; Vodusek et al. 1986) and, therefore, it has been used in patients with neurological disease or injury to inhibit undesired detrusor contractions. It relies on activating spinal inhibitory systems that are capable of interrupting a detrusor contraction. Stimulation does not have to be applied continuously but conditional stimulation, ie only when a detrusor contraction occurs, is at least as effective (Dalmose et al. 2003; Kirkham et al. 2001).

However, for conditional stimulation to be feasible as a treatment option a safe and reliable method for chronic monitoring intravesical pressure is needed. Artificial sensors often cause problems in a chronic setting (Koldewijn et al. 1994), but with the advent of methods for long-term electrical interfacing with nerves, recording from natural sensors in the human body has become a realistic alternative (Sinkjaer et al. 1999).

Earlier studies showed that intravesical pressure (or wall tension) is the primary stimulus for activation of pelvic afferents in the cat (Habler et al. 1993) and rat (Sengupta and Gebhart 1994) by recording an increase in single nerve fiber activity with increased tension in the bladder wall and bladder pressure. More recently, whole nerve electroneurographic (ENG) signals reflecting changes in bladder pressure were recorded using cuff electrodes placed on the pelvic nerves and sacral nerve roots in cats (Jezernik et al. 2001) and pigs (Jezernik et al. 2000). Subsequent chronic implants in pigs showed that afferent ENG signals originating from bladder, rectum and cutaneous mechanoreceptors can also be recorded with implanted electrodes (Kurstjens et al. 2001).

We investigated whether afferent nerve activity related to mechanical activity of the bladder and other pelvic organs can be recorded using cuff electrodes placed on an extradural sacral nerve root in human. Nerve activity was intra-operatively recorded in SCI-patients who underwent implantation of a sacral root stimulator for bladder and bowel control.

2 Materials and Methods

The study was approved by the local ethical committee of Institut Guttman, and informed consents were obtained.

2.1 Surgical preparation

Nerve recordings were performed under general anesthesia (1% Propofol 40-50 ml/h and Remifentanil 0.15-0.25 µg/kg/min) in 6 SCI-patients (Table 1) who underwent implantation of an extradural FineTech-Brindley Bladder System. During the operation, access to the extradural sacral nerve roots was gained by a laminectomy (L5 to S4) and the individual roots were identified anatomically and by the response of different muscle groups to electrical stimulation with a hook electrode. Bipolar silicone cuff electrodes (inner diameter 2.6-3.8 mm, length 10 mm, 25 µm thick platinum foil ring contacts 1 mm width, reference contact on the outside of the cuff) were placed on the extradural sacral root showing the largest bladder response to stimulation in patients 1-3, and on the contralateral root in patients 4-6. The area around the cuff was filled with saline (body temperature), and the cuff electrode was connected to a sterile telemeter (Donaldson et al. 2003). Nerve signals were then recorded for 30-45 minutes, where

after the cuff electrode was removed and the normal surgical procedure was resumed. The experimental setup is shown in fig. 1.

Patient	Gender, age	Level and severity of injury	Cause of and time since injury	Cystometric Capacity [ml]
1	Male, 43	C6, Incomplete	Myelitis, 17 years	269
2	Female, 45	T3, Complete	Trauma, 6 years	83
3	Female, 27	C5/6, Complete	MVA*, 7 years	100
4	Male, 20	C5, Complete	Diving, 3 years	191
5	Male, 49	T3, Complete	MVA*, 3 years	258
6	Male, 35	T5, Complete	MVA*, 2 years	283

*MVA: motor vehicle accident.

Table 1. Patient data.

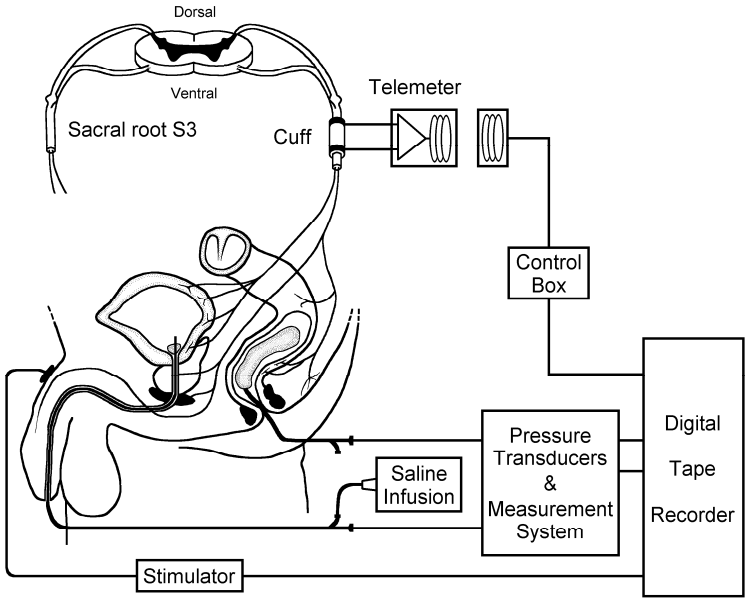

Fig. 1: Schematic overview of the experimental set-up.

2.2 Urodynamic monitoring

Rectal and intravesical pressures were measured through fluid filled lines connected to a pressure recording system (type 21C15 manometers, DISA, Denmark). Intravesical pressure (Pves) was measured through a single lumen 5Ch catheter, while rectal pressure (Prect) was measured through a 10Ch rectal balloon catheter mounted against a 18Ch distension catheter. The measurement balloon was positioned at the midpoint of the distension balloon. The pressure signals and the signal from the telemeter were stored on digital tape (RD-135T DAT data recorder, TEAC) for offline analysis.

2.3 Experimental protocol

Sensory compound action potentials - Electrical stimulation of the dorsal penile/clitoral nerve was performed to test the neural interface. Two self-adhesive surface electrodes (PALS, Axelgaard Co., USA) were placed 2 cm apart on the dorsum of the penis in males. In females, one electrode was placed over the clitoris and the other suprapubic. CAPs were elicited with monophasic pulses (1-50 mA, 200 μs, 6 Hz) using a custom-made stimulator.

Stimulation of the dermatome - ENG was recorded when mechanically stimulating (tapping and stroking) the relevant sacral dermatome by hand.

Rectal distension - The rectum was distended by consecutive 50 ml saline bolus injections into a rectal balloon, made of a condom mounted on a single lumen 18Ch catheter.

Bladder filling - To fill the bladder, a 3-lumen 18Ch transurethral catheter was inserted, and 50 ml saline boluses were infused consecutively until a volume of 400 ml was reached or Pves exceeded 100 cmH2O.

Bladder contractions - In 5 of the 6 patients, the contra-lateral sacral root was electrically stimulated with a hook electrode (10 V, 350 μs, 30 Hz) to evoke bladder contractions while simultaneously recording ENG.

2.4 Signal processing and data analysis

All signals were re-sampled at 20 kHz and stored on a PC. CAPs were averaged to reduce noise, the peak-to-peak amplitude was measured, and the onset latency was measured as the time to the first peak (Buchthal and Rosenfalck 1966). The ENG signals were band-pass filtered (300-3000 Hz, 10th order, zero phase lag Butterworth filter) and the variance of the ENG and the time average of the pressure signals were calculated per time bin (40 ms for cutaneous ENG and 100 ms for bladder and rectal ENG).

The signal-to-noise ratio (SNR) of ENG responses was calculated as the ratio between the variances of the ENG during peak neural activity and the background noise, corrected for the level of background noise (1 s average for bladder and rectal responses). Student's t-test was used to detect statistical significant ($p<0.05$) increase in ENG. The cross correlation coefficient (r) between ENG and bladder and rectal pressure respectively was calculated for a period including 2 s before and after each infusion.

3 Results

3.1 Compound Action Potentials

Sensory CAPs were present in all 6 patients. Figure 2 shows an example of CAP responses to different stimulation amplitudes. No response was present at 1 mA, but at 10 mA a small response was evoked. With increasing stimulation amplitude more fibers were activated, resulting in CAPs with larger amplitude and altered shape.

The onset latency of the averaged CAP responses to supra-maximal stimulation ranged from 4.0 to 8.4 ms, while the onset latencies in the two female patients (4.0 and 4.4 ms) were shorter than in the four male patients (5.8-8.4 ms). The mean peak-to-peak response to supra-maximal stimulation was 7.4±5.2 μV, ranging from 4.0±1.5 μV to 17.6±1.1 μV across all patients.

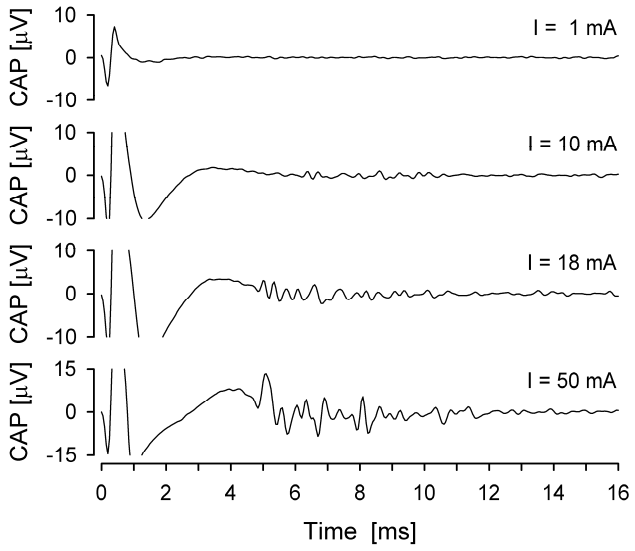

Fig. 2: Compound action potentials elicited by electrical stimulation of the dorsal penile nerve and recorded from a sacral root in patient 3. Stimulation artefact is present from t=0-4s.

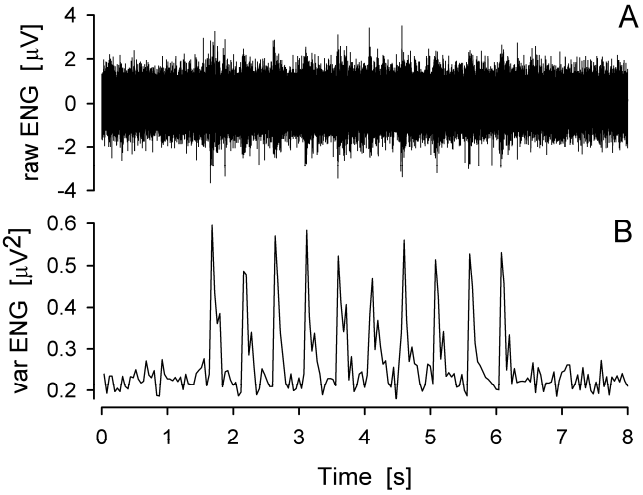

Fig. 3: Nerve responses recorded during ten skin taps in patient 6: **(A)** changes in the raw ENG were small. **(B)** Large increases in the ENG variance marked the onset of skin contact for each tap. The secondary, smaller response following the initial peak in 8 out of the 10 tap responses represents the neural response to the release of the skin impression made during the taps.

3.2 Nerve recordings during dermatome stimulation

Nerve responses during stroking and tapping the S3 dermatome were present in all 6 patients. Tapping evoked clearer ENG responses than stroking. The responses in the raw signal were small and often just above the noise level. Figure 3 shows ENG responses to skin taps in patient 6. The neural response evoked by each tap could barely be distinguished from the noise in the raw signal (fig. 3A). However, the neural responses are clear after processing the signal (fig. 3B).

The amplitude of ENG responses evoked by tapping ranged from SNR = 1.43±0.41 (n=20) in patient 1 to 5.25±0.98 (n=20) in patient 3, with a mean SNR of 3.06±1.65 across all patients.

3.3 Nerve recordings during rectal distension

Neural responses to rectal distension were recorded in all 6 patients. In the first three patients, the distension balloon was filled with 3-4 subsequent 50 ml bolus infusions. But, because in two of these patients Prect did not exceed 15-27 cmH2O and increases in ENG signal were not significant or had a low correlation (-0.2<r<0.2), the balloon was made approximately 50% smaller and infused with 6-8 boluses instead. Following this, peak pressures of 68-104 cmH2O were obtained but ENG responses remained small and mainly phasic.

Figure 4 shows a recording from patient 5. Small phasic ENG responses were most evident during the last 2 out of 7 infusions, and a small tonic response was also observed. After bolus 7, phasic ENG responses were evoked by repetitive withdrawal and fast re-infusion of 50 ml.

During such repeated infusions, peaks of 60±4 (n=3, patient 2) to 97±3 (n=4, patient 5) cmH2O were obtained in Prect by pressure increases ranging from 18±2 (n=5, patient 4) to 67±1 (n=5, patient 1) cmH2O. ENG responses varied from 0.06±0.01 (n=5, patient 6) to 0.30±0.07 (patient 4), and cross correlation coefficients ranged from 0.33±0.09 (patient 6) to 0.69±0.07 (patient 4).

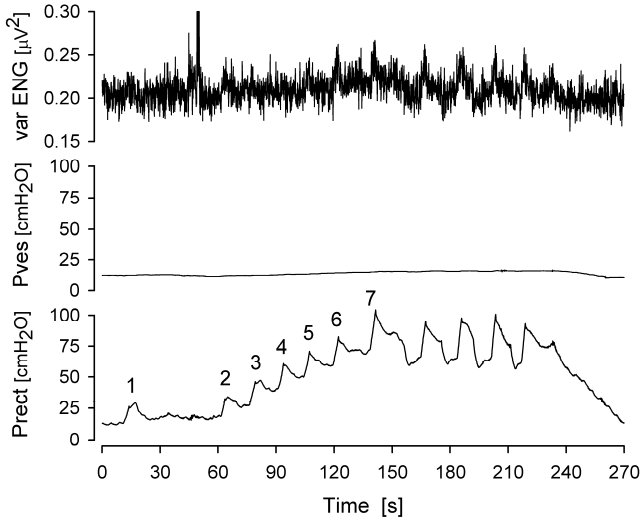

Fig. 4: Nerve activity and pressure response recorded during rectal distension in patient 5. A small phasic ENG response was already present during the first bolus infusion. The series of infusions was shortly halted after the 1st infusion because of interference in the signal received from the telemeter (at approximately t = ±50 s, marked by an out-of-scale artefact).

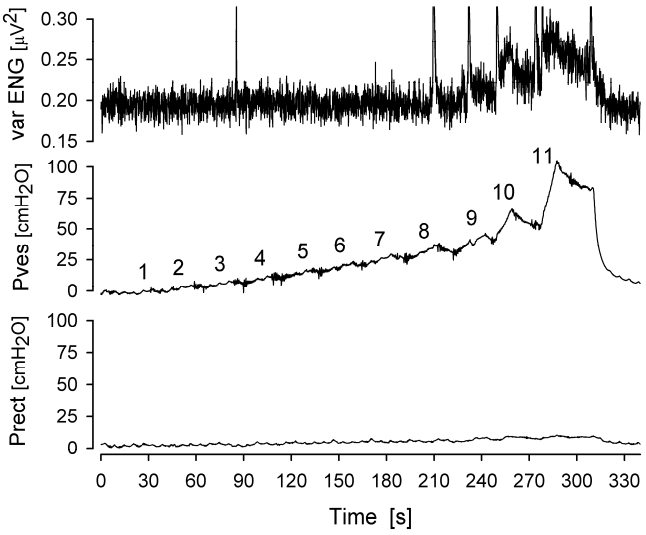

Fig. 5: Nerve activity and pressure response recorded during artificial bladder filling in patient 4. The numbers 1-11 indicate when each of the boluses was infused. The short lasting and out of scale peaks in ENG activity were probably due to afferent activity from the urethra and skin evoked by movement of the filling catheter during infusion.

3.4 Nerve recordings during bladder filling

In 5 out of 6 patients ENG responses were present during bladder filling. In patient 1, eight consecutive 50 ml infusions failed to increase Pves and no changes in nerve activity were observed. A typical recording is shown in Figure 5. During the first seven infusions, Pves gradually increased to 34 cmH2O but no changes in nerve activity were observed. With the last four infusions Pves increased further and significant increases in ENG were recorded. Peaks in the ENG were reached before the pressure peaked. Following each infusion Pves decreased and, depending on the pressure after each infusion, a tonic ENG response was maintained.

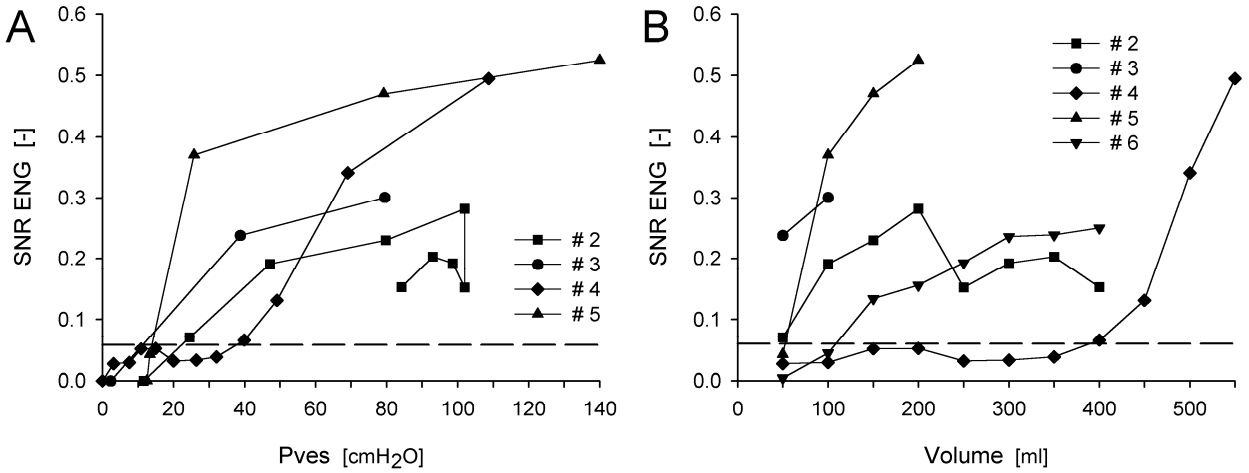

Fig. 6: Overview of the ENG responses recorded during bladder filling: **(A)** SNR of peak ENG responses versus peak intravesical pressure, and **(B)** SNR of peak ENG responses versus total infused volume after each subsequent 50 ml bolus infusion. Responses below the dashed horizontal line were not significant ($p>0.05$). Pves was not measured in patient 6 because of technical problems.

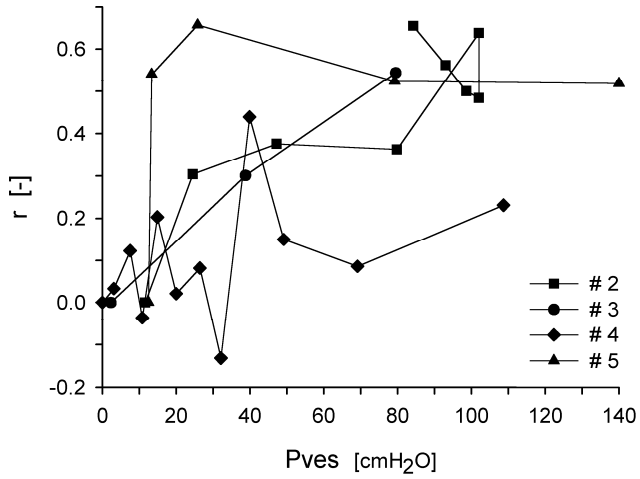

Fig. 7: Cross correlation (r) between Pves and ENG responses recorded during bladder filling. Mainly phasic nature of the ENG responses compared with pressure profile kept cross correlation low. In patient 4, it was even negative during two infusions.

Figure 6 shows the SNR of the ENG signals for each subsequent infusion as function of the peak intravesical pressures (fig. 6A), and as function of the infused volume (fig. 6B). In 2 out of 5 patients there was a significant increase in ENG ($p<0.004$) with the first infusion, but for the total group the first significant increase in ENG was obtained with the third infusion (Vinfused = 150±146 ml, n=5) where a peak Pves of 32±8 cmH2O (n=4) was obtained.

The correlation coefficients for the changes in Pves and ENG signals for each subsequent infusion are shown in fig. 7. A tendency is present that the correlation increases with increasing intravesical pressures. Although in general clear increases in ENG signal were present for Pves > 30-40 cmH2O, the correlation with Pves was low and in one case even negative.

An example of the large phasic versus tonic component in the relation between ENG and Pves is shown in fig. 8. Following the third infusion, an increase in the tonic component as function of Pves was present. This threshold in Pves for tonic response ranged from 25 to 40 cmH2O (n=4).

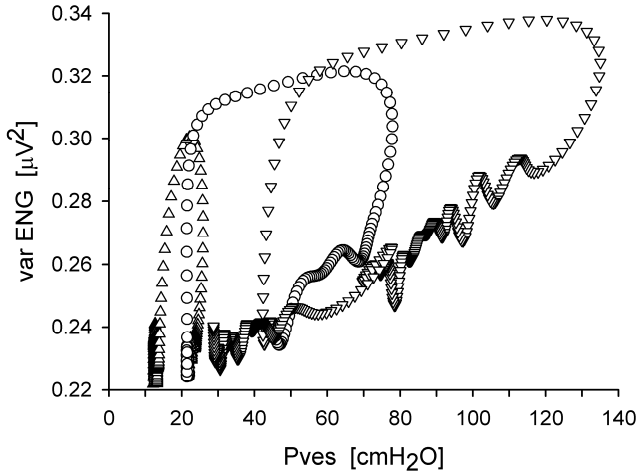

Fig. 8: patient 5. Relationship between recorded nerve signal and intravesical pressure during consecutive bolus infusions. Only responses to the second (Δ), third (O) and fourth (∇) infusion are shown since no significant increase in ENG and Pves was recorded during the first infusion.

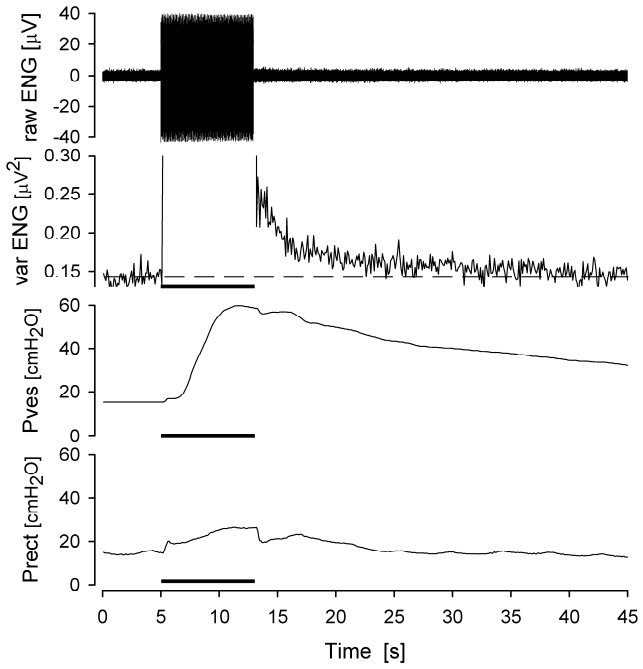

Fig. 9: Nerve signal recorded during a bladder contraction evoked by electrical stimulation (t = 5-13 s, indicated by the horizontal bars) of the contra lateral sacral root in patient 5. Pves increased to 60 cmH_2O at t = 11 s and decreased only gradually after stimulation. Stimulation artefacts saturated the nerve amplifier (raw ENG, top trace) and therefore also the variance signal (var ENG, second trace). The amplifier recovered within 15 ms of each stimulus. The variance signal however recovered from saturation only one time bin (100 ms) after stimulation was stopped, but still showed an increase that rapidly decreased as the bladder relaxed. The dashed horizontal line in the second trace indicates the level of background neural activity prior to the onset of stimulation.

3.5 Nerve recordings during bladder contractions

An increase in ENG during a bladder contraction evoked by electrical stimulation of the contra lateral sacral root was present in 4 out of 5 patients. Figure 9 shows pressure and ENG responses during one such stimulation. Stimulation artefacts saturated the ENG variance off the scale shown, but an ENG response was present immediately after stimulation.

In 1 out of 2 stimulated patients with the cuff placed on the root with the largest bladder response to test stimulation, a Pves of 30 cmH2O and an ENG response of SNR = 0.12 were obtained. In the three patients with the electrode placed contralaterally, nerve responses with peak SNR of 0.47±0.27 (n=3) were observed following bladder pressures of 57±7 cmH2O (n=3).

4 Discussion

This study demonstrates the feasibility of recording afferent nerve activity originating from different pelvic organs using a cuff electrode placed on an extradural S3 sacral root in human. CAPs were recorded following electrical stimulation of the dorsal penile/clitoral nerve. The distribution of dorsal penile/clitoral afferents over the S1-S3 nerve roots is however asymmetric with the majority of fibers being carried by S2 (Huang et al. 1997), which could account for the relatively low CAP amplitudes recorded in this study. The onset latencies of CAPs in the male

patients were longer than in the females, likely due to the longer distance between recording and stimulation sites since the electrodes were placed on the base of the flaccid penis (Yang et al. 1997).

The ENG responses to mechanical stimulation of the dermatome, bladder and rectum were small but characteristic for nerve cuff recordings (Sinkjaer et al. 1999). The aggregate ENG recorded with a cuff electrode depends on the number of active fibers, is dominated by the large diameter fibers, is biased in favour of superficial axons, and is larger in amplitude for smaller cuff diameters (Hoffer and Kallesøe 2001). The cuff diameters used in this study were relatively large compared to previous animal studies (Jezernik et al. 2000; Jezernik et al. 2001), thus lower signal amplitudes were expected. Furthermore, cutaneous ENG responses were larger than ENG from the bladder and rectum as the cutaneous mechanoreceptors are innervated by large myelinated (Aβ) fibers while visceral mechanoreceptors are innervated by small myelinated (Aδ) and unmyelinated (C) fibers.

Consistent ENG responses were observed in all patients where bolus infusions increased the rectal or bladder pressure. Nerve responses to rectal distensions were smaller than during bladder fillings. This might be because the increases and absolute values of Prect during distensions were generally smaller than during bladder fillings, and only a small part of the rectum was pressurized. Furthermore, rectal afferents are probably distributed over several sacral roots and not just the one we recorded from. Nerve responses during rectal distension were mainly phasic, but during bladder fillings a tonic component was also recorded. The cross-correlation between nerve responses and bladder or rectal pressures was therefore low, despite a significant increase in ENG.

Jezernik et al observed mainly phasic responses in an animal model (Jezernik et al. 2000). The present study shows however that with a sufficient high static pressure (Pves > 40 cmH2O), a tonic nerve response is also present. Bladder pressure during the storage phase varies from 7 to 20 cmH2O and increases under isovolumetric conditions to about 34 to 41 cmH2O when normal, non-painful micturition commences (Janig and Morrison 1986). The patients in our study had lower bladder capacities than normal and were under general anesthesia, but the latter pressure range mentioned is similar to the threshold of the tonic nerve response.

The amplitude of recorded nerve signals increases when using a smaller diameter cuff electrode. This would be possible by placing the cuff on the intradural dorsal nerve root. Furthermore, in this study nerve responses were recorded during mechanical stimulation of the bladder, rectum and skin separately, and under general anesthesia. In clinical application however, the neural signal is a mixture of contributions from nerve fibers innervating these and other afferent sources. Sensory information from the bladder can only be recovered from this compound signal if the contribution from bladder afferents is sufficiently large, or if bladder afferents have such unique signal properties that they can be distinguished from other afferents. Recording pelvic nerve activity would increase selectivity (Jezernik et al. 2000). However, in contrast to a single and anatomically distinct nerve in the previous animal model (Wen et al. 1999), the human pelvic nerve splits early in its course through the pelvis, forming a plexus unsuitable for cuff electrode application (Rijkhoff et al. 1997). Optimal filtering techniques have shown to improve the SNR and selectivity of ENG signals from individual afferent sources (Jezernik and Grill 2001), but their effectiveness on a compound signal is unknown.

5 Conclusions

This study shows the feasibility of recording afferent nerve activity from the bladder using a cuff electrode placed on the extradural S3 sacral root in human. The phasic nature of the nerve responses favours the application to detect bladder contractions, but monitoring bladder volume will be more difficult because of the high threshold for a tonic response. The small amplitudes of neural signals and the various other sources that contribute to the sacral nerve root ENG indicate that improvements in recording quality and more sophisticated signal processing methods are needed to reliably detect bladder contractions in a chronic application.

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Chapter 5

Classification of whole nerve activity using signal-dependent wavelets.

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In short-paper form published in: Proceedings of the 9th Vienna International Workshop on Functional Electrical Stimulation, 19-22 September, 2007, Krems, Austria. Bijak M., Mand T., Martinek J., May ,W. and Pichler, M. (eds.). Vienna: Medical University of Vienna, Vienna Medical School, Center for Biomedical Engineering and Physics, pp. 183-186.
In abstract form also published in: Artificial Organs 31(8): p. A23.

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Abstract
Traditional nerve cuff electrodes record an aggregate signal and thus provide only one channel of information. However, since most peripheral nerves innervate multiple structures, greater selectivity is needed if nerve signals are to provide sensory feedback or control signals in neuroprosthetic devices. In this study we apply a pattern recognition approach based on signal-dependent wavelets to classify sacral root nerve signals generated from mechanical stimulation of the bladder, rectum and skin. Nerve signals from the skin were discriminated from those from bladder and rectum with misclassification rates ranging 29.4-34.5% and 23.3-36.5% respectively. Discrimination between nerve signals from the bladder and rectum was more difficult (40.1-44.4% error). Doubling the length of signals used decreased the classification error for all pairs of classes (bladder-rectal: 36.6-43.8%; bladder-skin: 24.5-28.2%; rectal-skin: 12.8-28.8%). The results indicate that signal projection on optimized wavelet functions is promising for discrimination between different types of sacral root sensory nerve signals of relative short duration. Future work should include a more sophisticated classifier and extension to multi-class comparisons.

1 Introduction

Nerve cuff electrodes are extraneural electrodes that have been used for the recording of peripheral nerve activity since the early 1970s (Hoffer et al. 1974; Stein et al. 1975). Cuff electrodes are typically small tubes made of a silastic material and contain one or more electrically conducting contacts on the inside. The circumferential placement of cuff electrodes has several advantages: the amplitude of the recorded nerve activity signal increases as a result of a restriction of the extracellular space, and interference by extraneural signals can be reduced by using an appropriate contact configuration. Furthermore, cuff electrodes can be implanted safely, providing a stable neural interface for long-term recording of neural signals (Stein et al. 1977; Struijk et al. 1999).

Recording sensory nerve signals from peripheral nerves has therefore become a realistic alternative to artificial sensors to provide feedback or control signals in neuro-prosthetic devices (Hoffer et al. 1996; Sinkjaer et al. 1999). Neural signals are used in current neural prosthesis systems to correct foot drop (Haugland and Sinkjaer 1995) and restore hand grasp (Inmann et al. 2001). In these applications, onset or changes in skin contact is detected from increases in nerve activity recorded from distal and purely cutaneous nerves that innervate only a defined skin area. In other applications, electrode implantation on more proximal nerves may be necessary because of e.g. anatomical reasons or safer electrode application. For example, in case of a neural prosthesis to treat neurogenic bladder overactivity in patients with spinal cord injury, the sacral nerve roots are of interest for chronic electrode application (Rijkhoff et al. 1997). As the sacral nerve roots provide a combined sensory and motor innervation of multiple end organs, similar increases in whole nerve activity can be recorded when activating mechanoreceptors in the bladder, rectum or relevant skin areas(Jezernik et al. 2000; Jezernik et al. 2001a; Kurstjens et al. 2005). In a chronic application, information from the bladder can therefore only be extracted if the contribution from bladder afferent fibers can be distinguished from other contributing sources.

Although cuff electrode recordings provide only a single channel of information (Hoffer and Kallesøe 2001), a certain degree of selectivity might be available depending on the geometry of the electrode contacts and composition of the nerve. For a given cuff geometry, both the amplitude and the frequency spectrum of the recorded signals depend on the conduction velocity and thus diameter of the nerve fibers (Stein et al. 1975; Struijk 1997). Different signal components can therefore be enhanced using appropriate filtering techniques (Jezernik and Grill 2001), and the spectral properties of the whole nerve signals can be used for classification (Jezernik et al. 2001b). However, the frequency bandwidths of the signal components in whole nerve recordings largely overlap. This only allows classification of these signal components when they do not overlap in time.

As the recorded nerve activity is a summation of signals of compact support (the nerve fiber action potentials) that occur at random time intervals, traditional Fourier transformation using sine bases can lead to a poor representation, and other basis functions that use compact support waveforms may be preferred. The discrete wavelet transform (DWT) is an example of transformation in compact support functions, called wavelets. In the present study, a pattern recognition method based on DWT (Maitrot et al. 2005) was applied to nerve signal classification.

2 Methods

2.1 Acquisition of nerve activity

Whole nerve activity was intra-operatively recorded from the extradural S3 sacral roots in spinal cord injured patients who underwent implantation of an extradural FineTech-Brindley Bladder System (Kurstjens et al. 2005). Access to the sacral roots was gained by laminectomy. Bipolar cuff electrodes were placed unilateral on an extradural S3 sacral root and connected to a telemetric device. Nerve activity was recorded during excitation of mechanosensitive afferent nerve fibers originating from the bladder (rapid saline bolus infusions), rectum (rapid balloon dissentions), and skin (tapping and stroking the appropriate dermatome by hand). The signal received from the telemeter was stored on digital tape for later offline analysis.

In this study, only data recorded from two representative patients were used. Following re-sampling from tape (20 kHz sample rate), the signals were first stored on a personal computer before further analysis. The signals were then band-pass filtered in the frequency range 300 Hz - 3 kHz (10th order, with a zero phase lag Butterworth filter). Signal portions exceeding 2*SD the mean baseline activity were then divided into signal epochs of 512 samples. Three classes of nerve signals were formed according to their origin: bladder (B), rectum (R) and skin (C, cutaneous). Table 1 provides an overview of the number of signals in the data sets of different classes used for training and validation.

	Patient 1		**Patient 2**	
	Training	*Validation*	*Training*	*Validation*
Bladder	420	336	472	448
Rectum	120	80	240	200
Cutaneous	120	120	60	60

Table 1. Number of signal epochs collected in the different classes.

2.2 Discrete wavelet decomposition

The wavelet transformation is a decomposition on a set of basis functions, formed by dilations, contractions, and time shifts of one unique function of compact support. For a given signal x(t), the continuous wavelet transform (CWT) is defined as:

$$CWT(a,\tau) = \int x(t)\psi^{*}_{a,\tau}(t)\,dt$$

where $\psi a,\tau(t)$ is a set of wavelets and * denotes complex conjugation. The wavelet transform is an inner product operation that can be seen as a measure of similarity, or cross correlation, between the signal x(t) and each individual wavelet from the set $\psi a,\tau(t)$, which is obtained by scaling and translation of the basis function (or mother wavelet) $\psi(t)$:

$$\psi_{a,\tau}(t) = \frac{1}{\sqrt{a}}\psi\left(\frac{t-\tau}{a}\right)$$

where a represents the scaling factor and τ the translation in time.

The CWT is a redundant representation of the signal. A non redundant, still invertible, representation is obtained by sampling the parameters (a, τ). Varying the resolution parameters exponentially results in a partial scale-invariant representation and when at the same time sampling on a dyadic grid (base 2) (Mallat 1989), the discrete wavelet is given by

$$\psi_{j,k}(t) = 2^{\frac{-j}{2}} \psi(2^{-j} t - k) \qquad j,\ k \text{ integer}$$

where j controls the dilation of the mother wavelet in dyadic scaled resolutions. The DWT of a discrete signal x(n) is then obtained as:

$$DWT(j,k) = 2^{\frac{-j}{2}} \sum_n a^x_{2^j}(n)\ \psi(2^{-j} n - k)$$

where $a^x_{2^j}(n)$ is the approximation of *x(n)* at scale 2^j and is obtained by convolving *x(n)* with a scaling function $\phi(x)$ which has the same resolution (scale) as the discrete wavelet. According to the multiresolution analysis (MRA) framework (Mallat 1989), the scaling and wavelet functions satisfy the two-scale equations:

$$\phi(x/2) = \sqrt{2} \sum_n h(n) \phi(x - n)$$

$$\psi(x/2) = \sqrt{2} \sum_n g(n) \phi(x - n)$$

where h(n) and g(n) are the scaling and wavelet filter coefficients, which are the impulse response of respectively a discrete low-pass and high-pass filter. In case of orthonormal wavelets, g(n) can be obtained from h(n):

$$g(n) = (-1)^{1-n} h(1 - n)$$

The entire decomposition is thus obtained from h(n). To generate orthogonal wavelets in a multiresolution space using a finite impulse response (FIR) filter of length L, there are L/2+1 conditions to fulfil (Lawton 1990; Lawton 1991). After first ensuring the existence of ϕ and orthogonality of ψ, there remain L/2-1 degrees of freedom that can be brought under control via unconstrained optimisation (Vaidyanathan and Hoang 1988). For a filter h(n) with L coefficients this is accomplished using a set of trigonometric functions, as given in e.g. (Selesnick 1997), that are input with a design parameter vector θ containing (L-2)/2 angles.

2.3 Definition of feature space

The selected signals of each class with length N were decomposed on the discrete wavelet basis described previously, using Mallat's algorithm (Mallat 1989) and the implementation by (Maitrot et al. 2005). The obtained N wavelet coefficients cx(j,k) were normalized to make the representation space insensitive to the wave-form occurrence time instants, and the normalized marginals of the DWT were calculated as:

$$m_x(j) = \sum_{k=0}^{N/2^j - 1} d_x(j,k) \qquad j = 1, \ldots, J$$

$$d_x(j,k) = \frac{|c_x(j,k)|}{\sum_{j=1}^{J} \sum_{k=o}^{N/2^j - 1} |c_x(j,k)|}$$

where $J = \log_2(N)$ is the deepest level of decomposition. The vector $\mathbf{F}_m = [m_x(1), \ldots, m_x(J)]$ provides information on the distribution of the wavelet coefficients over *J* bands, giving a representation of the energy distribution over different dyadic scaled frequency bands when using a particular wavelet shape. Different mother wavelets were constructed by varying the angle(s) in the vector θ from -π to +π using a grid of π/30, for both the one-component (1D, with θ = [α]) and two-component (2D, with θ = [α β]) version.

2.4 Classification of nerve activity

As the focus of this study was the application of wavelet parameterization optimised to the signal characteristics, only a relatively simple classifier was used. Let ωi be the training set of signals for the class Ωi, with i = 1,…, I, where I is the total number of classes. The representative $R_{\omega i}^{F}$ of class ωi was calculated as the average vector of the feature spaces Fωi computed from the training signals of that class. For each signal x of ωi, classification of the signals was based on the rule of nearest representative:

assign x to ω_i if $i = \inf_I d\left(F_{x,\omega i}, R_{\omega i}^{F}\right)$

where d is the distance between the feature spaces. Two types of distance measures were tested: Euclidian and Kullback distance. The Euclidian distance between the feature vector of a signal and the representative of its class is given by:

$$d_E(F_{x,\Omega}, R_\Omega) = \sqrt{\sum_{j=1}^{J}\left[F_{x,\Omega}(j) - R_\Omega(j)\right]^2}$$

and the Kullback distance is given by:

$$d_K(F_{x,\Omega}, R_\Omega) = \sum_{J=1}^{J}\left[F_{x,\Omega}(j)\log\frac{F_{x,\Omega}(j)}{R_\Omega(j)} + R_\Omega(j)\log\frac{R_\Omega(j)}{F_{x,\Omega}(j)}\right]$$

The probability of classification error, estimated from the training set of signals, was used as quality criterion to optimise the classification. The error probability for a class ωi, $P_e^F(\omega_i)$, is the total number of misclassifications divided by the number of signals in ωi, while the overall error probability of classification is found by averaging the probability of all classes:

$$P_e = \frac{1}{I}\sum_{i=1}^{I} P_e^F(\omega_i)$$

The overall classification error on the training set was minimized by optimising the design parameter θ such that it corresponds to a mother wavelet that projects the signals in the best discriminating feature space. For optimization, only the training set was used. The misclassification rate was then computed from the validation set (no common elements with the training set).

3 Results

3.1 Single component design parameter

Figure 1 shows the probability of classification error estimated for the learning sets when attempting to separate nerve signals (L = 512) from the bladder and rectum of patient 1 based on the Euclidian distance between feature spaces. With using only one component (α) for the design parameter θ, the misclassification can be seen to vary significantly for different mother wavelets constructed by α. The value of α resulting in a minimum of the average of the two error probabilities defines the optimal wavelet for decomposition of this particular training set, which was then used for classification of the signals in the validation sets to obtain the final error probability. Classification was also performed for the data set pairs of bladder-cutaneous (B-C) and rectum-cutaneous (R-C), and for the same signals but with length 2L (but for only N/2 signals), see table 2A. Results obtained when using the Kullback distance between feature spaces for the same classifications are given in table 2B.

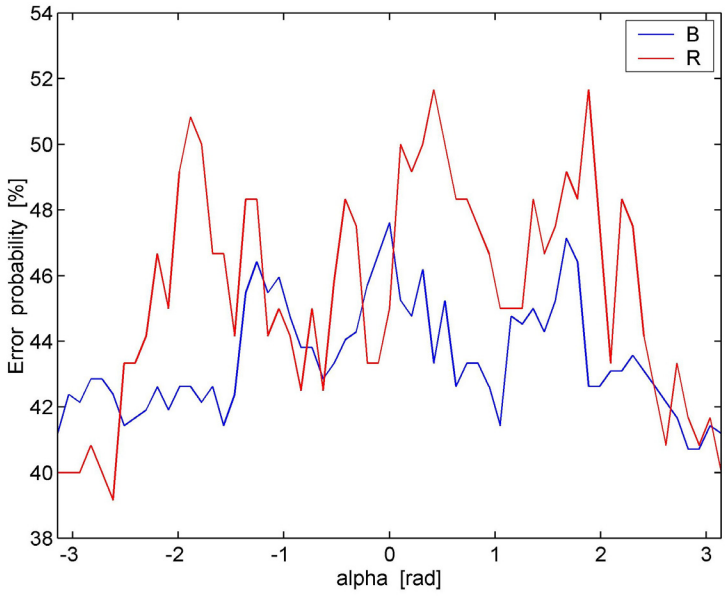

Fig. 1: Misclassification rates estimated from the learning sets for different values of the single component design parameter $\theta = [\alpha]$ when using the Euclidian distance measure to classify nerve signals as originating from the bladder (B) or the rectum (R) in patient 1.

A	**Patient 1**		**Patient 2**	
	L = 512	*L = 1024*	*L = 512*	*L = 1024*
B – R	41.6 (40. 6)	36.3 (35.4)	44.4 (43.4)	41.8 (40.4)
B – C	33.4 (28.9)	27.5 (22.3)	34.0 (23.5)	24.6 (11.8)
R – C	30.8 (28.3)	24.2 (21.2)	23.3 (20.6)	12.8 (9.6)

B	**Patient 1**		**Patient 2**	
	L = 512	*L = 1024*	*L = 512*	*L = 1024*
B – R	40.1 (38.9)	36.9 (34.5)	42.4 (43.3)	44.0 (40.5)
B – C	31.9 (27.1)	25.0 (21.0)	29.4 (20.5)	25.6 (15.6)
R – C	36.5 (25.8)	28.8 (16.7)	26.5 (17.7)	19.0 (12.1)

Table 2. Misclassification rates in percent for classification of the nerve signals in the test (training) sets when using the single component design parameter $\theta = [\alpha]$ for construction of the mother wavelet and classification based on the Euclidian **(A)** and the Kullback **(B)** distance between feature spaces.

The misclassification rates obtained for the training sets were lower than for the validation sets in both patients. Furthermore, the misclassification rate decreased when increasing the length of the signal, and thereby increasing the resolution of the wavelet decomposition. Finally, although in most cases the lowest misclassification rates on training set were obtained using the Kullback distance, the final misclassification rate on the validation sets was often smaller when the Euclidian distance measure was used.

3.2 Two-component design parameter

Enlarging the design parameter to a two-component vector $\theta = [\alpha\ \beta]$ increases the complexity of the mother wavelets, increasing the variation in misclassification rate obtained for different values of θ (Figure 2). This lead to lower minimum probabilities of misclassification in all training sets, see table 3. However, when the optimal training values on the validation sets, no improvement was found compared to the results when using a single component θ only.

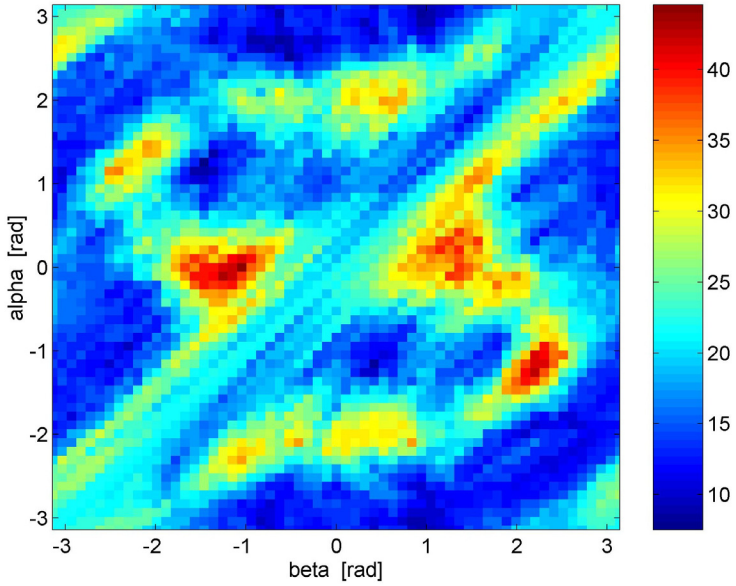

Fig. 2: Average probability of misclassification [%] estimated from the learning sets for nerve signals (L = 1024) from the rectum and skin of patient 2, and using the Kullback distance measure.

A	**Patient 1**		**Patient 2**	
	L = 512	*L = 1024*	*L = 512*	*L = 1024*
B – R	44.6 (37.8)	43.3 (35.0)	41.3 (41.4)	38.9 (38.7)
B – C	34.5 (28.0)	27.6 (18.5)	32.2 (19.2)	28.2 (9.7)
R – C	29.8 (25.0)	23.8 (17.5)	27.3 (17.1)	19.5 (7.9)

B	**Patient 1**		**Patient 2**	
	L = 512	*L = 1024*	*L = 512*	*L = 1024*
B – R	41.6 (27.8)	37.9 (32.1)	42.5 (41.3)	41.7 (35.9)
B – C	35.7 (26.0)	27.7 (17.4)	33.1 (17.3)	24.5 (11.4)
R – C	32.7 (24.2)	25.0 (14.2)	25.3 (15.2)	24.5 (7.5)

Table 3. Misclassification rates in percent for classification of the nerve signals in the test (training) sets when using the two-components for the design parameter θ when constructing the mother wavelets and classification based on **(A)** the Euclidian distance, or **(B)** the Kullback distance between feature spaces.

4 Discussion and Conclusions

The signal-dependent wavelet classification method was originally developed for classification of surface electromyographic (EMG) signals and was based on the observation that these signals are composed of compact support waveforms, which are scaled depending on the conduction velocity of intracellular muscle action potentials and on the depth of the muscle fibers forming the motor unit (Lindstrom and Magnusson 1977; Maitrot et al. 2005). Signals recorded from cuff electrodes show a large resemblance to this observation as they depend on the geometry of the cuff electrode and the nerve action potential conduction velocity, are dominated by the activity of the largest diameter fibers, and are biased in favor of superficial fibers (Hoffer and Kallesøe 2001). This resemblance suggested that wavelet decomposition could also provide a natural representation for neural signals recorded from cuff electrodes and therefore might form a suitable feature space for classification.

The results in this study demonstrate that sacral root nerve signal recorded during activation of mechanoreceptors in the bladder, rectum and sacral dermatome can be classified based on a feature space obtained by the discrete wavelet transformation. The optimal mother wavelets were found for a set of training signals, adapting the feature space to the characteristics that discriminated best between the sensory origin of the nerve signals. However, validation of the optimal wavelets on a second set of signals showed a larger misclassification probability. Although the test and validation data sets were assumed to contain signals from the same source, the actual signals were different and the information content was therefore not exactly the same. Furthermore, even despite the fact that the marginals were calculated using normalized wavelets coefficients, the misclassification rate seemed consistently smaller for signals with larger difference in SNR when comparing the results from patient 1 with patient 2 and nerve signals from the bladder/rectum versus the cutaneous nerve signals. Although some degree of cross talk cannot be excluded because both organs are mechanically connected, the poor classification rate could therefore also suggest that both organs are innervated by population of nerve fibers with similar diameter distributions.

In a previous study using sacral root nerve signals, classification was performed based on the autocorrelation function of nerve activity recorded during a time period of 1.25 second (Jezernik et al. 2001b). The current study demonstrates that this can be reduced considerably by using wavelet decomposition for classification. We used signals with durations of respectively 0.0256 and 0.0512 second. Further doubling the signal length would decrease the obtained probability of misclassification more whilst still ten times shorter signals are needed.

Not all decomposition levels were effectively used in creating a feature space because information was removed from certain decomposition levels as a result of band-pass filtering the recorded nerve signals before wavelet decomposition. The feature space contains more information when using non-filtered data, which could reduce the probability of misclassification. Alternatively, other characteristic feature spaces based on methods such as average absolute coefficients, scaling energy or maximum, singular value decomposition or auto regression coefficients (Zhang et al. 2005) could also be tested. Further improvements might also be obtained when using more sophisticated methods for classification instead of the distance measures used in this study, for example a cluster separation index (Davies and Bouldin 1979), Bayesian decision rules (Mallet et al. 1997), artificial neural networks, or using a more sophisticated adaptive wavelet algorithm (Mallet et al. 1997).

Finally, only two-class comparisons were made in the present study, but when classifying sensory signals recorded from peripheral nerves such proximal as the sacral roots, it is important to consider all sources the neural activity could originate from. Future work should therefore include an extension to the multi-class case, also allowing a direct comparison of the signal-dependent wavelet method with previous classification methods.

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Chapter 6

Discussion and Conclusions

1 Summary of the main findings and conclusions

This thesis concerns the recording of sensory neural signals from peripheral nerves that innervate the urinary bladder. From these signals, information on the mechanical activity of the bladder can be extracted. The ultimate goal is to use the neural signals originating from the natural sensors in the bladder for close-loop control in an implantable neuroprosthesis for treatment of neurogenic detrusor overactivity in patients with a spinal cord injury. The studies presented in this thesis aim to extend earlier work towards the realization of such a method.

The questions addressed in this thesis can be divided into three main subjects: implantation of the cuff electrode in a chronic animal model (questions 1&2), validation of the results obtained in animals in a human study (question 3), and test of a recently developed method for signal classification for its ability to identify the sensory origin of recorded nerve activity (question 4).

1. *Is it possible to implant cuff electrodes chronically on the sacral roots for the purpose of recording of sensory nerve activity?*

Cuff electrodes were implanted on the extradural sacral root in pigs and the state of the neural interface was evaluated on a regular basis. Nerve sections were taken after conclusion of the implants and examined for possible histological changes. The duration of implantation varied across animals from 19 days to more than one year. Infection was found not be of a problem with this kind of implantations, but the results showed that the success mainly depended on the amount of damage that was inflicted to neural structure during or after electrode implantation. The measures used to access the state of the neural interface were amplitude and latency of evoked CAP responses, the SNR and the median frequency of cutaneous nerve activity. They all showed to be good indicators for changes in the neural structure and correlated well with the histological findings. The extradural sacral root was found to be very susceptible for nerve damage because of the limited space available for the cuff electrode. One implant proved successful implant for more than one year (including several additional surgeries). This is a strong indication that when the space problem is avoided, long-term implantation of a cuff electrode for recording of sacral root sensory nerve signals is feasible.

2. *What kind of signals can be recorded from a cuff electrode chronically implanted on a sacral nerve root and how do they relate to specific physiological events, in particular mechanical activity of the bladder?*

Nerve activity was recorded from chronically implanted cuff electrodes during bladder filling, rectal distension and mechanical stimulation of the sacral dermatome in pigs.
During initial experiments, in which the animals were anesthetized, nerve responses from the bladder and rectum had much lower amplitude than nerve responses from the skin. Furthermore, and it was mainly only during changes (onset/release for dermatome contact and increase in pressure for bladder and rectal distension) that responses were present.

Cutaneous ENG responses recorded in the conscious pig did not differ much from those recorded when the pig was anesthetized, but the recording of afferent ENG from the rectum and bladder specifically was more difficult. Nerve activity recorded during the conscious cystometries correlated well with the pig's voiding behavior in general, but it did not reflect the mechanical activity (change of pressure) of the bladder itself. An increase in nerve responses during manual squeezing of the bladder in one animal showed that bladder afferent nerve activity can be recorded from a cuff electrode chronically implanted on a sacral root after more than one year of implantation. However, the contribution from the bladder afferents to the aggregate activity during natural behavior was too small to obtain a correlation between bladder pressure and the RBI processed whole nerve signal.

3. *Is it possible to record afferent nerve activity related to mechanical bladder activity by means of cuff electrodes placed on sacral nerve roots in human?*

A cuff electrode was temporarily placed on an extradural S3 sacral root in spinal cord injured humans undergoing surgical implantation of a FineTech-Brindley bladder system. Using an experimental protocol nearly identical to the pig study (see chapter 3), it was demonstrated that also in human increases in whole sacral root nerve activity can be recorded in response to mechanical stimulation of the relevant dermatome, rectal distension, rapid bladder filling and bladder contraction evoked by electrical stimulation of the sacral nerve root.

The mainly phasic nature of the recorded nerve responses was similar to those recorded in the chronically implanted pigs. In addition, while the first phasic nerve responses were recorded when the intravesical pressure exceeded a pressure level of approximately 20-30 cm H_2O in both human and the anesthetized pig, a tonic nerve response was also present when between infusions a static pressure greater than approximately 40 cm H_2O was maintained in the human.

4. *Is it possible to identify the recorded nerve activity according to its sensory origin?*

The problem of identifying the correct origin of sensory whole nerve activity was investigated in chapter 5. Supervised classification of afferent nerve activity from the bladder rectum and skin was performed based on a feature space obtained from a discrete wavelet transformation of the neural signal. Mother wavelets were optimized to obtain maximal discrimination between the sensory origins of the activity. Using nerve signals recorded from two SCI-patients, it was found that cutaneous afferent nerve signals could be distinguished from nerve activity from the rectum and the bladder with a minimum error of respectively 12.8% and 24.6%. The distinction between signals from the rectum or bladder was found to be much more difficult (minimum 36.6% error).

These results indicate that the rectum and bladder are innervated by afferent nerve fibers with similar conduction velocity properties while those innervating the skin are more different. This is

consistent with literature on conduction velocities from single sacral root afferent nerve fibers in human (Schalow and Lang 1989). The results are however inconsistent with results by (Jezernik et al. 2001b) where the nerve conduction velocity of fibers innervating the bladder were found to be more close to those innervating the dermatome rather than those innervating the rectum. This inconsistency is believed to be due to the difference in species.

Furthermore, the classification errors obtained in the present study were slightly larger than obtained previously ((Jezernik et al. 2001b) but the results are nevertheless encouraging because considerable shorter (ten times) signal epochs were used for classification.

2 Methodological considerations

2.1 Animal model

The pig was used as animal model in this thesis because its anatomy and innervation are similar to those in human and the storage and voiding function are well described (Dalmose et al. 2000; Guan et al. 1994; Mills et al. 2000). Mini pigs were used for chronic implantation because of their moderate size and their slow increase in size over long periods of time. A pig model was originally also used in the first acute animal study on bladder related nerve cuff recordings by (Jezernik et al. 2000). Although consistent nerve responses were recorded during rapid bolus infusions in the acute pigs, nerve responses during slow bladder filling were recorded in less than half of the number of animals investigated and no satisfactory bladder contractions could be induced or observed. When the animal model therefore was changed to cats instead, slow bladder filling led to quasi-periodic bladder contractions, during which an increase in mainly S1 nerve activity was strongly correlated with the bladder pressure (Jezernik et al. 2001a). These quasi-periodic contractions at moderate volume are normally absent in the conscious cat (Klevmark 1980), and are thought to result from the anesthesia affecting some critical inhibitory synapses in the neural pathways regulating bladder mobility so as to make it hyperactive (Morrison 1987). Although unnatural, this behavior may serve as a good model for studies related to NDO. On the other hand, as it is only artificially induced behavior, this cat model is unsuitable when considering chronic implant of cuff electrodes for recording spontaneous nerve activity in the conscious animal.

The chronic implant study was started by implanting the cuff electrodes on the extradural sacral root that showed the largest increase in bladder pressure when electrically stimulated with a hook electrode. This method of selection was based on the assumption that the nerve root with the strongest efferent innervation would also contain the best afferent innervation, although no information about this was found in literature. As a result, the cuffs were implanted on different roots in different pigs. The majority was implanted on the S2 and S3 nerve roots, which is consistent with the innervation of the bladder in pigs (Nickel et al. 2004). The nerve responses that were recorded during bladder fillings were mainly obtained from both S2 and S3. This in contrast to the previous acute study in pigs where, when recording the nerve activity from both the S2 and S3 sacral roots, a nerve response was only obtained from the S3 root in half of the investigated animals (Jezernik et al. 2000).

The S1 root is in general not indicated to innervate the bladder in pig, but very small nerve responses were recorded during bladder filling. Nerve responses recorded during the conscious cystometries did then also not resemble bladder activity but were associated with activity from the urethral sphincter, which does receive innervation from S1. Activity of the bladder, on the

other hand, is controlled through the pelvic nerves which are mainly formed from the S2 and S3 sacral roots. But unfortunately nerve damage and other technical problems prevented the recording of nerve activity during conscious activity in the pigs with cuff electrodes implanted on those particular nerve roots.

2.2 Evaluation of the neural interface

The courses of the measures used to access the status of the neural interface were in general consistent with each other. The SNR of cutaneous ENG during conscious recordings was however more sensitive to changes in noise and background nerve activity because of the absence of anesthesia, a poor radio link and residual EMG contamination. The amount of EMG was kept at a minimum by using the tripolar recording configuration (Andreasen et al. 2000; Stein et al. 1975) in combination with off-line high-pass filtering at 800 Hz (Gordon et al. 1980; Popovic et al. 1993). The high-pass frequency was later reduced to 400 Hz (chapter 3) because most of the power in the bladder ENG frequency spectrum is well below the 800 Hz (Jezernik and Grill 2001). No filtering was necessary for the CAP responses since the averaging process eliminated all other signals that were not synchronous to the stimulation.

2.3 Histological evaluation of the implanted nerves

In previous cuff implant studies mainly dealing with implantation on the sciatic, tibial or peroneal nerve, control sections were taken from nerves in the contra-lateral leg (Stein et al. 1977), controls or sham-operated animals (Larsen et al. 1998). In the present study, both the implanted and contra-lateral nerve were located within the same anatomical area and therefore imbedded in a post operative grown block of connective tissue. This block could only be removed as a whole specimen from the spinal canal without identification of individual nerve roots. The implanted root was then identified afterwards when the cuff electrode became visible during cutting of the tissue sections for histological processing while the contra-lateral root remained unidentifiable.

Hematoxylin and eosin (H&E) was used for staining the nerve sections. H&E is useful to studying the general morphology but it does not stain lipids and may therefore not be the most useful stain for examining axons and myelin. Other stains such as toluidine blue can provide more information on this matter and may have been more been useful when control sections were available for comparison of e.g. myelin thickness and fiber diameter distributions. Nevertheless, a good indication of the thickness of myelin sheaths was still obtained based of the voids left after it was removed by the histological processing.

2.4 Nerve signal processing

One of the first applications of natural sensory feedback in human involved the use of cutaneous nerve activity for close-loop control of paralyzed muscle (Haugland 1994). Assuming that information about skin contact force was contained within a frequency band equal to the one of the force signal, the envelope of raw cutaneous ENG was obtained by rectification and bin integration (RBI) with a time constant appropriate for the force signal. RBI has also the advantage that the contribution of noise to the signal bin is reduced by the averaging while the contribution related to nerve activity is maintained. Since then, rectification and bin integration or low-pass filtering has been the traditional method for processing nerve cuff signals. In chapter 3, RBI was therefore used for quantification of the nerve activity. The results showed however that the contribution from the bladder afferents to the recorded signal was often so small that it

many times stayed below the level of detection, and therefore, to increase sensitivity in the human study, RBI processing was replaced by measurement of the variance. The variance of the raw nerve signal is directly proportional to the number of active nerve fibers whereas RBI processing of a nerve cuff signal results only in a signal that is proportional to the square root of that number (Jezernik and Sinkjaer 1999).

The data used in the study on signal dependent wavelet signal to classify the sensory origin of the nerve signals was chosen from these particular two patients based on the amplitude of their nerve response compared rest of the group: small, but not the smallest, and large, but not the largest. Also, it was only human data that was used without further testing animal data to keep it as close as possible to clinical application. Although a further generalization was made by normalizing the wavelet coefficients, the misclassification rates were consistently different. This was probably because the recorded signals did not consist of nerve activity only but for a large part of noise.

2.5 Results in animal model versus results in human

The experimental protocol used to activate mechanoreceptors in the bladder, rectum and sacral dermatome was similar in both the human and the pig study. The main difference was that the cuff electrodes used in the humans were bipolar in stead of tripolar. This was done because results from the pig implant had shown that the nerve length available extradurally is limited and almost no EMG contamination or external interference signals were expected because of the intraoperative setting. Furthermore, larger diameter cuff electrodes had to be used because the human sacral nerve roots were larger. Smaller nerve responses were thus expected, but the nerve responses recorded from the dermatome and bladder in the humans were much large than those recorded in the pigs. There were several factors that could have contributed to this difference. Firstly, besides the afore mentioned use of variance in stead of RBI calculation, recording from a bipolar electrode configuration results in larger amplitude of recorded nerve potentials than from a tripolar configuration (Stein et al. 1977). But on the other hand, the population of activated large diameter myelinated nerve fibers could also have been larger in the human subjects.

Furthermore, the electronic input circuitry of the telemeter used in the human study was modified to have a reduced frequency pass band than the versions fabricated until then. The design of the telemeter was based on an amplifier developed earlier for recording of sensory nerve signals from the skin (Haugland and Sinkjaer 1995). As a consequence, one of the design criteria was that the passband should be from 800 Hz to 8 kHz (Donaldson et al. 2003). However, the nerve fibers innervating the bladder have much lower conduction velocities and therefore the frequency spectrum of the recorded neural signals is lower as well (Jezernik and Grill 2001). This partial mismatch in frequency spectrum may therefore have been one of the contributing factors in the low nerve responses recorded from the bladder in the chronic pig study in this thesis.

Another factor, in case of recording from the bladder, was the level of intravesical bladder pressure obtained with artificial fillings. In the anesthetized pig, only small nerve responses were recorded when Pves exceeded a pressure level of approximately 20-30 cmH_2O. Similar thresholds for nerve response were also seen in the human study but the main difference was that during fillings in the human subjects the bladder pressure arose always far above this threshold while in the pig no much larger pressures were reached.

Finally, because of the type of injury in SCI patients, the protocols used for anesthesia and analgesia are different than during surgery in patients without such injury. The level of

anesthesia is reduced after the initial surgical procedure (laminectomy) to access the sacral roots to attain clearer central and peripheral responses to electrical stimulation of the nerve roots, initially for correct root identification and later for the selective rhizotomy. Thus, with the intra-operative recordings performed between these two moments, a reduced suppression of the nervous system likely contributed to the recording of a larger amount of bladder afferent nerve activity as compared to in the anesthetized pigs.

3 Future aspects and recommendations

Results obtained in the human study in this thesis exceeded all expectations based on previous animal studies utilizing cuff electrodes for recording of nerve activity. While the feasibility of recording an increase in sacral root nerve activity related to mechanical bladder activity now also has been demonstrated in human, the similarity of the nerve responses to those recorded in the animal models demonstrate the main shortcoming: lack of selectivity towards bladder related afferent nerve activity. This shortcoming results from the low sensitivity of a cuff electrode to signals from small diameter nerve fibers and the fact that the sacral roots are mixed nerves with a relative large diameter. Improvements may be obtained by more advanced signal processing techniques, a different location of electrode application or improved electrode design.

3.1 Signal processing

The results from the signal dependant discrete wavelet classification were very encouraging, considering the length of the signal epochs and simple classifier used. Both the present study and the previous study by (Jezernik et al. 2001b) demonstrate that, to a certain extent, bladder afferent nerve signals can be identified from whole nerve activity recorded from the sacral roots. Future studies will however have to show how much the classification could be improved depending on the length of the signal epochs, use of more sophisticated classifiers and for multi-class classification, as well as include data from more human subjects. Finally, the performance of the classification methods on compound nerve activity has to be determined.

3.2 Location of electrode application

Optimization of signal processing techniques will improve selectivity towards bladder related signals, but the gain will most likely only be marginal because of the nature of the nerve activity that is recorded from a traditional cuff electrode. In stead, greater improvement may be achieved during the stage of recording the nerve activity. This includes choosing an alternative location for placement of the recording electrode, changing the design of the electrode itself or, probably the best, a combination of both.

Seen from the current location (the extradural sacral roots), alternative locations that may lead to improved signal recording can be found in opposite directions: more proximal within the dural sack or more distally towards the bladder. Intradural placement on the dorsal nerve roots has the advantage of limiting the recording to afferent nerve activity only and with the prospect of larger signals because of using smaller diameter cuff electrodes. In an ongoing study, nerve activity from the intradural S3 dorsal sacral nerve root has now been recorded in several SCI-patients but only the results of one patient has been published so far (Sinkjær et al. 2000). More distally, the pudendal nerve can be used to detect bladder contractions in case sphincter dyssynergia is

present (Wenzel et al. 2005). However, the issue of selectivity would still remains as at that point the pudendal nerve still contains its rectal, cutaneous, and dorsal penile/clitoral nerve branches.

3.3 Cuff electrode design

The largest improvement in selectivity may be obtained with an alternative cuff electrode design. Information on the direction of nerve conduction (i.e. efferent or afferent action potentials) is only obtained by recording mono and bipolar. In the popular (quasi) tri-polar recording configuration the end contacts are shorted to reject signals external to the cuff but indication of the direction of conduction is lost. Another kind of selectivity can however be obtained based on the velocity of conduction. From computer simulation and experimental studies it is known that by choosing the correct contact spacing the performance of recording from nerve fibers with a certain diameter can be improved (Andreasen and Struijk 2002; Stein et al. 1975; Stein et al. 1977; Struijk 1997). Furthermore, the cuff electrode can be regarded as a velocity dependent bandpass filter with a transfer function depending on the configuration of the electrode contacts (Struijk 1997; Struijk and Thomsen 1995). Based on time delays, summations and narrow bandpass filters, Taylor et al. (2004) proposed a theoretical method using multi-contact nerve cuffs to recorded ENG signals selectively by fiber group conduction velocity. Results from the first *in vitro* experiment demonstrated that it was possible to enhance the response of a fiber diameter group from an evoked compound action potential response (Rieger et al. 2004). Although the intradural dorsal sacral nerve root is probably physically the best location for application of such a rather long multi contact cuff electrode, success in a practical application will largely depend on the amount of afferent nerve activity from the bladder compared to all other nerve activity in that root on a given moment in time considering that the bladder afferents are only a small part of the afferent fibers that form the sacral root.

3.4 Clinical prospects

Despite the different issues that were of a concern in the animal studies so far, the prospects for chronic implant in human SCI-patients are positive. Anatomically, there is more space and free nerve length in the intraspinal canal which will ease handling of the nerve and reducing nerve damage, and offers possibilities for next generation cuff electrodes. Clinically, there is already a considerable amount of experience on chronic sacral root electrode implantation (the FineTech-Brindley Bladder System). Therefore, clinical feasibility will largely depend on the ability to record more selectively from a specific subpopulation of afferent nerve fibers associated with the bladder while rejecting activity from other nerve fibers and extra-neural interference signals.

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Dansk sammenfatning

Neurogen detrusor overaktivitet (NDO) er en almindelig form for blæredysfunktionalitet hos patienter med neurologisk betingede sygdomme eller rygmarvsskade. Sygdommen forårsager fejl i de nedre urinveje og den er karakteriseret ved ufrivillige blærekontraktioner ved en relativ lav volumen. Hvis NDO ikke bliver behandlet, kan det medføre lav blærekapacitet, inkontinens, høj intravesikalt tryk og refluks af urin hvilket kan forårsage skade på nyrerne. Konventionelle behandlingsmetoder er sjældent succesfulde og kan have alvorlige bivirkninger.

Alternativt kan NDO behandles ved hjælp af elektrisk stimulation af passende afferente nerve fibre. Herved aktiveres eksisterende, inhibitoriske nerve baner i rygmarven, der er i stand til at forhindre detrusor kontraktion. Dette princip medfører dog, at en sensor skal kunne detektere hvornår en kontraktion begynder. Tidligere studier baseret på akutte dyremodeller har vist at afferent nerve aktivitet, der er associeret med mekanisk aktivitet i blæren, kan optages fra manchet elektroder. Signaler opsamlet fra de perifere nerver der innerverer blæren kan altså bruges som en sådan sensor. Det primære formål med denne afhandling var at implantere manchet elektroder til optagelse af sensoriske nerve signaler fra blæren i en kronisk dyremodel, og at tage skridtet fra dyremodel til udførelse af det første præ-kliniske studie.

Manchet elektroder blev placeret omkring ekstradurale, sakrale nerverødder i mini-grise. Det neurale interface blev evalueret regelmæssigt ved evaluering af stimuleret nerve aktivitet samt kutan nerve aktivitet. Udvalgte nerve-sektioner blev efter terminering af forsøgene desuden undersøgt for mulige histologiske ændringer. Varigheden af implanteringen varierede fra 19 dage til mere end et år. Resultaterne viste at succes hovedsageligt afhang af størrelsen af den nerve skade der var sket under eller efter elektrode implanteringen. Den ekstradurale sakrale nerverod var især udsat for nerve skade på grund af den begrænsede plads til manchet elektroden. Dog fungerede et implantat succesfuldt i mere end et år, hvilket indikerer at kronisk implantering af en cuff elektrode til optagelse af sensoriske signaler fra sakrale nerve rødder er muligt.

Ved opfølgende forsøg blev nerve aktiviteten monitoreret før, under og efter mekanisk stimulation af den sakrale dermatom, blære fyldninger og rektale udvidelser. I begyndelsen af forsøgsrækken blev dyrene bedøvet, og det var muligt at registrere nerve response fra blære og rektum, men disse var meget mindre end nerve signaler registret fra dermatomer. I slutningen af forsøgsrækken blev der målt fra vågne dyr, og kutane nerve signaler var stadig til stede, men signaler fra rektum og især blæren var sværere at registrere på grund af øget baggrundsaktivitet fra nerven og muskel aktivitet. Nerve aktivitet registreret under cystometri korrelerede generelt med grisens vandladnings adfærd, men ikke med aktivitet af selve blæren, hvilket indikerer at bidrag fra blærens afferente nerve fibre til den samlede neurale aktivitet under almindelig adfær er for lille til at kunne udlede en sammenhæng imellem blære tryk og det samlede, registrerede nerve signal.

Et præ-klinisk studie blev gennemført for at undersøge aktiviteten i de sakrale nerverødder i mennesker. En manchet elektrode blev kort varigt placeret ekstraduralt omkring S3 sakral nerveroden i rygmarvsskadede patienter udtaget til at gennemgå en kirurgisk procedure til implantering af et FineTech-Brindley blære system. Der blev anvendt en eksperimentiel protokol der var næsten identisk til den anvendt i grise studierne. Det blev demonstreret, at den samlede

nerve aktivitet i den sakrale nerverod kunne registreres under mekanisk stimulation af blæren, rektum og en relevant dermatom.

I et pilot-studie blev kontrolleret klassifikation anvendt til at separere den afferente nerve aktivitet fra henholdsvis blære, rektum og hud optaget fra to rygmarvsskadede patienter. Klassificeringen blev baseret på parametre fra en diskret wavelet transformation af det neurale signal. Resultatet viste at de kutane nerve signaler kunne separeres fra signaler fra rektum og blære med en fejl på henholdsvis 12.8% og 24.6%, men det var vanskeligere at separere signaler fra de sidste to kilder fra hinanden (36.6% fejl).

Resultaterne opnået igennem arbejdet i denne afhandling viser, at når man tager de rette forhåndregler, så er det muligt at optage afferent nerve aktivitet fra forskellige bækken organer ved brug af kronisk implanterede manchet elektroder omkring de sakrale nerverødder. Derudover blev det demonstreret at sammenlignelige resultater kunne opnåes i akutte dyre modeller og i mennesker. Men resultaterne fra de to modeller viste også den samme svaghed; manglende selektivitet til at opfange aktivitet fra blæren i nerve signalet. Forbedringer er nødvendige før en egentlig klinisk applikation er muligt, hvilket kan opnås igennem anvendelse af mere avancerede signal processerings teknikker, valg af en anden implanterings lokation for elektroden eller igennem forbedret elektrode design.

List of publications

Journal papers:

Kurstjens G.A.M., Dalmose A.L., Rijkhoff N.J.M., Sinkjær T.: Electroneurographic signals from sacral roots in pigs using long-term implanted cuff electrodes. *To be submitted*.

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