Urinary Bladder Innervation Within the Sacral Roots of a Sheep*

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***Abstract*— Managing the urinary bladder after spinal cord injury is of primary importance because neurogenic dysfunction leads to life-threatening complications. Sacral Anterior Root Stimulators that control the bladder have been available for many years, however, these devices cannot sense the fullness of the bladder or detect the onset of reflex voiding. In order to address this fundamental limitation, this paper explores the possibility of recording the neural signals that encode bladder fullness from the sacral roots in sheep using extra-neural books. Stimulation of and recording from six roots (S1, S2 and S3 bilaterally) shows that efferent and afferent pathways seem to be co-located within roots, but also that simultaneous recording from multiple roots may be useful to enhance overall signal quality.**

I. Introduction

Historically, kidney damage following Spinal Cord Injury (SCI) was usually fatal, and so the management of the urinary bladder after SCI has become essential. Restoration of normal bladder function is also frequently rated as a high priority by people living with SCI, because it strongly affects quality of life [1]. In order to avoid kidney damage, and to improve quality of life, both the storage and emptying functions of the bladder need to be effective.

At present the most common method for emptying is intermittent catheterisation; this is expensive, invasive, and infection prone [2]. As an alternative, for complete-lesion patients a *neuroprosthesis* for controlling the bladder was developed by Giles Brindley some 40 years ago [3]. This is currently manufactured by Finetech Medical Ltd (UK) and approximately 4,000 devices have been implanted worldwide.

The Brindley device employs Sacral Anterior Root Stimulation (SARS) to control the muscles (detrusors and sphincters) of the urinary bladder. During implantation surgery, a *rhizotomy* of the sacral posterior roots is usually performed because this improves urine flow while voiding but also prevents reflex incontinence, improving the storage function. This is unpopular because it inflicts further trauma to the nerves and removes remaining sexual function.

The need for a rhizotomy arises because as the bladder fills it undergoes reflex contractions that are normally inhibited by the brain. With SCI these contractions are not blocked, and spontaneous voiding occurs unless the posterior roots are cut. An improved neuroprosthesis is thus required that can sense and stop this reflex voiding, removing the need for a rhizotomy. This might be done by recording and decoding of the electrical signals (the electroneurogram – ENG) within the sacral roots that encode bladder pressure and fullness. We are investigating the feasibility of this approach.

Existing research has shown that it is possible to record neural signals that encode bladder fullness from both the pelvic nerve and the sacral roots using an implantable cuff electrode [4][5]. Signals recorded from the pelvic nerve have a greater amplitude than those recorded from the sacral roots, although these amplitudes are still very low. However, accessing the pelvic nerve is difficult and may not be straightforward in humans. The sacral roots meanwhile are easily accessible via a laminectomy of the sacral vertebra; the same procedure is how the Brindley device is currently implanted in humans.

It is strongly desirable to only implant electrodes on one root, or as few roots as is necessary. Electrical stimulation of the individual roots, concurrent with flow cystometry, can identify which root contains the most bladder efferents. However, it is not clear which of the sacral roots will contain the greatest number of bladder afferents, or if these roots are the same as those with the greatest efferent response. Thus, this presents a significant challenge in identifying which roots should be used for stimulation and recording.

This paper presents preliminary results from acute in-vivo experiments performed in sheep, wherein three electrode *books* were implanted on S1, S2 and S3 bilaterally. The goal is to identify which roots are the best target for selective recording of bladder afferents in a chronic implant.

II. Methods

A. Surgical Approach

All the experimental procedures detailed in this paper were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 under Home Office regulations.

An acute experiment was performed on an adult female sheep (weight 63 kg). The ewe underwent general anaesthesia involving: a pre-operative transdermal fentanyl patch, induction with IV ketamine (7.5 mg/kg) and IV midazolam (0.5 mg/kg), and maintenance on inhaled sevoflurane mixed in oxygen. Intraoperative fentanyl & ketamine analgesia were provided as required. A lumbosacral dorsal laminectomy was performed through the midline to expose the extradural sacral roots and each root was identified based on anatomical landmarks (e.g. lumbosacral junction) and by their responses to electrical stimulation. At the end of the experiment the animal was terminated by overdose of pentobarbital.

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B. Cystometry

Cystometry was performed using a clinical cystometry system (Medica S.p.A PicoSmart) that enabled simultaneous infusion of sterile saline and pressure measurement *via* a two-lumen 7Fr silicone catheter placed in the bladder. A 12Fr Foley catheter was also placed, the balloon inflated and the catheter pulled back and held against the bladder neck to prevent leaking of fluid during filling. In all cases the fill rate was 50 mL/min and bladder pressures were limited to 50 cmH_2O maximum. In order to identify the autonomic function of the roots, the bladder was filled before each root was stimulated using monophasic square wave pulses at 30 Hz with amplitudes ranging from 1 – 30 V using an adjustable and battery powered surgical stimulator (Finetech Medical Model BSD260).

C. Electrodes

The recording interfaces comprised three custom-made *books*, manufactured by Finetech Medical (UK). Each book was made from silicone and contained two slots, embedded within each slot were three U-shaped platinum-iridium electrodes (spacing 3 mm) (Fig. 1). The books were designed so that they could rest on top of the spinal canal, with each pair of left and right roots being placed in each of the two slots before sealing with Kwik-Sil (World Precision Instruments) (Fig. 2). Books were made with varying slot sizes in order to accommodate intraspecific variation in root diameter. The electrical connections to the books were established via implantable *Cooper* cables terminated with mini-XLR plugs. Each book had two 3-core Cooper cables corresponding to each slot. Stimulation electrodes were bipolar extradural surgical probes (Finetech Medical BSC259). Each root was stimulated by holding the root in the hooks.

D. Recording

The implanted books were connected to custom-made amplifiers as dipoles, with each dipole formed between successive electrodes, for a total of two dipoles per slot. The amplifiers are based on commercial instrumentation amplifiers (AD8429 – Analog Devices). The overall amplifier gain was 80 dB and the system bandwidth was 100 Hz – 50 kHz. The input referred noise floor with the inputs shorted was approximately 0.7 μV RMS per channel. This corresponds to a passband input-referred noise density of about 3.27 nV/√Hz. The expected amplitudes of the action potentials recorded with a book are very low (typically 1 – 10 μV for single action potentials and 10 – 100 μV for compound action potentials).

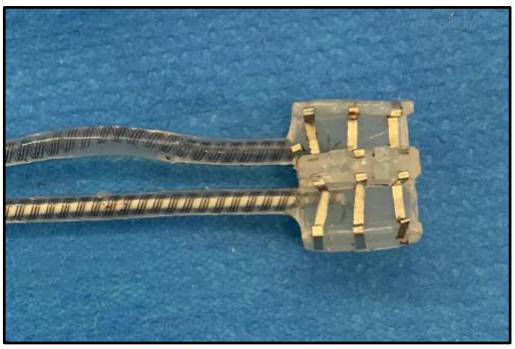

Figure 1: An example of a three-electrode book used in this experiment. Each book contained two slots, each with three electrodes. Cooper cable was used to connect the electrodes to the amplifiers.

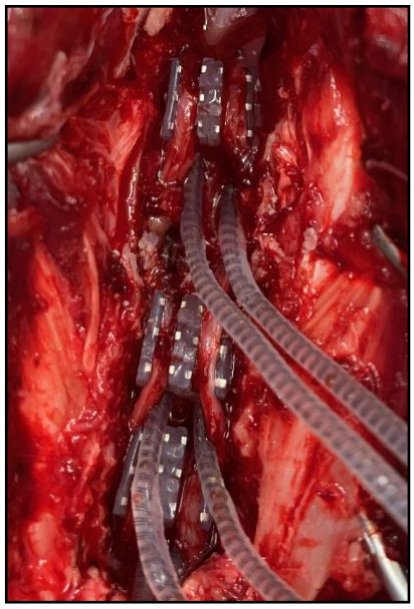

Figure 2: Three books implanted in the spinal canal (top of the photograph is the cranial aspect of the animal) with S1-S3 bilateral roots in each slot. The Cooper cables run caudally to the amplifiers.

The amplified and filtered signals were digitised simultaneously using a bank of analogue to digital converters (NI9222 mounted in cDAQ-9178 by National Instruments, Austin, TX, USA) at a sample rate of 500 kS/s with 16-bit resolution. The high sampling rate (approximately 50 times the Nyquist frequency for the accepted signal bandwidth of 10 kHz) was chosen for convenience only and all data were down sampled to a new sample rate of 50 kS/s after recording. The electrodes were connected in a bipolar fashion (and the amplifier reference was connected to the epidermis *via* the retractors present in the surgical field.

E. Data Analysis

Data analysis was performed offline using MATLAB in order to extract bladder related information from the raw ENG recordings and to remove intermittent interference artefacts (short spikes, likely caused by accidentally knocking the Cooper cable during the recording). Each of the 12 channels was band-pass filtered (10 Hz – 10 kHz) before artefact rejection was performed by comparing each signal with the long term mean and standard deviation on a sample per sample basis. If a sample exceeded a pre-set threshold (mean ±3.5 the standard deviation) that sample was replaced with one equal to the long-term mean. In order to identify slowly changing features within each channel that might correlate with bladder activity a 200 ms moving variance window was computed [4]. Finally, the resulting variance waveforms were normalised in order to remove offsets caused by differing baseline signal amplitudes in each channel.

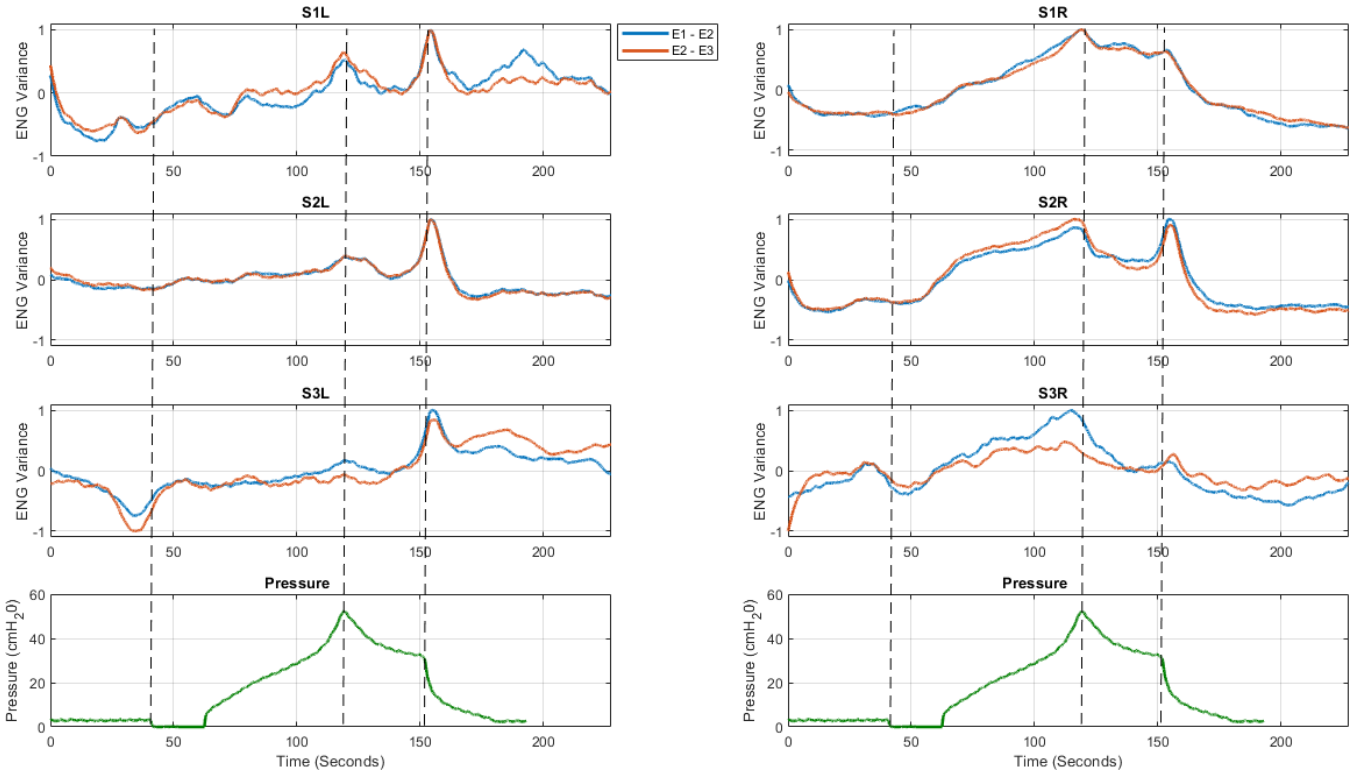

Figure 3: Bladder pressure and detected ENG variance (normalised, plotted as the bipoles formed by the first-second and second-third electrodes in each slot) in the six sacral roots. The dashed lines from left to right indicate pump on, pump off and opening of the catheter to drain the bladder from left to right respectively. All roots show some ENG response at the point of maximum pressure, with the clearest correlation to the cystometry pressure profile on the Right roots. All roots also show strong ENG responses to the opening of the catheter, albeit with some time delay. The pressure is plotted twice (left and right bottom graphs) for presentation purposes only.

III. Results

A. Bladder Function During Stimulation

Pre-operative and post-operative cystometries confirmed the ability to obtain bladder pressures of at least 50 cmH_2O by infusing, on average, 100 mL of fluid into the bladder. Some vaginal discharge and possible mastitis were noted pre-operatively. In all cases, once the pump had been stopped the bladder accommodated and the pressure fell to a stable level that was significantly below the peak pressure. The bladder was left with ~100 mL of saline infused during the surgical approach with no apparent loss of bladder muscle tone or pressure throughout the approach. Once the roots had been exposed the bladder was drained and then filled with 60 mL of saline to give a resting pressure of approximately 30 cmH_2O.

Each root was then stimulated individually by rapidly ramping the stimulation voltage to 20 V before holding at this level for five seconds. The stimulation results are shown in Table I and show that in all roots, stimulation gave rise to an increase in bladder pressure, but that the strongest efferent innervation of the urinary bladder was in S2 and S3 with a focus on the right-hand side roots. The darker shading indicates a greater increase in bladder pressure as a result of stimulation. The stimulation voltage applied to S1L was limited to 10 V because excessive tail movement and muscle activation of the leg extensors occured.

B. Electrode Impedances

The books were then implanted on S1, S2 and S3 bilaterally. Electrode impedances were measured in two-wire mode between electrodes 1 - 2 and 2 – 3 in each slot of each boot at 1 kHz using a 100 mV compliance voltage. The results shown in Table II highlight that the impedances were consistent across all of the electrodes at approximately 2 kΩ. The impedances were stable throughout the experiments. Once implanted the baseline signals recorded from each bipole were consistent at approximately 1.4 µV RMS, *i.e.* about double the input-referred noise floor of the amplifiers.

TABLE I. Bladder Response to Stimulation of the Root

Root		Stimulation Response			
		Starting Pressure (cmH_2O)	End Pressure (cmH_2O)	Increase (cmH_2O)	Stimulation Voltage (V)
S1	Left	13.1	27.1	14	10[a]
	Right	15.7	35.4	19.7	20
S2	Left	16.1	39.2	23.1	20
	Right	13.7	48.5	34.8	20
S3	Left	15.6	40.3	24.7	20
	Right	9.5	43.8	34.3	20

a. Voltage limited due to excessive tail movement and posterior muscle activation

C. Nerve Recordings

Once the books had been placed, the bladder was filled from empty at 50 mL/min (starting at T = 40s) and concurrent recordings were made from all electrodes. The recordings were then processed using the methods described in Section II. During bladder filling the ENG variance in all channels and the bladder pressure rose in tandem to a peak pressure of 50 cmH_2O (Fig. 3).

Once the peak pressure had been reached, the cystometry pump was stopped (T = 120s) and the bladder was allowed to accommodate, during which time the pressure slowly fell before stabilising at around 30 cmH_2O. Finally, the catheter was opened to void the bladder (T = 150s). Throughout the experiment the intra-channel ENG variance correlation was very high, in the range 99.6% (S1R) to 90.8% (S3R) as indicated in Table II.

The response to filling (level of afferent innervation) was measured as the increase in the normalised ENG variance from when the pump was turned on (T = 40s) to when the pump was turned off (T = 120s). As indicated in Table II, the largest increase in ENG variance was seen in S1R, S2R, and S3R. Darker shades indicate a larger increase. This is in broad agreement with the corresponding results in Table I for efferent innervation. Clearly visible in all roots was a large jump in the ENG variance that was nearly coincident (delayed by a few seconds) with the opening of the catheter.

In most channels the ENG variance returned to the starting level once the bladder was empty, however in some cases (e.g S1R) there was a 50% difference between the starting and ending ENG variance. Furthermore, there were changes in the ENG variance before bladder filling commenced, often with similar magnitudes to those seen during filling.

TABLE II. ENG PROPERTIES DURING FILLING

Root		ENG		
		Increase in Variance	**Intra-Channel Cross-Correlation**	**Impedance at 1 kHz (kΩ) (1 – 2 / 2 -3)**
S1	**Left**	0.83	92.00%	1.8 / 1.8
	Right	1.36	99.60%	1.9 / 2.5
S2	**Left**	0.43	99.00%	2.5 / 2.8
	Right	1.35	98.60%	1.9 / 1.9
S3	**Left**	0.42	92.00%	1.9 / 1.4
	Right	1.2	90.80%	1.8 / 1.9

IV. DISCUSSION AND CONCLUSIONS

It appears that there is some level of efferent bladder innervation within all of the roots, and in the single animal presented in this study this innervation appears to be stronger on the right-hand side roots. Likewise, it appears to be possible to record neural signals that encode bladder pressure from the extradural sacral roots using simple *book*s. This result is in line with early work performed in pigs, and more recent work using cuffs in sheep, and in all cases the apparent change in ENG variance is very small [5][4]. The afferent innervation appears, in this experiment, to match the efferent innervation in that the roots with the strongest stimulation response also produce the largest change in ENG variance during bladder filling.

In all roots there is an observed jump in the ENG variance shortly after opening the catheter to drain the bladder. It is not known if this is an afferent response from mechanoreceptors in the urethra or an efferent response in the form of a sacral reflex. Further work will involve trying to identify the origin of this response by selectively cutting the roots each side of the books. We will analyse larger datasets recorded from multiple animals following the same experimental process, and this work is on-going.

It remains to be seen if whole-nerve recordings from the sacral roots can detect reflex voiding, although the jump in the ENG variance when the catheter is opened suggests this may be possible, and if the same recording can be made in a freely moving animal. However, this paper has identified that there appears to be some correlation between the efferent and afferent responses of the sacral roots, and that bladder pressure information is encoded in *multiple* roots. In order to improve overall Signal-to-Noise Ratio (SNR) a future neuroprosthesis for management of the neurogenic urinary bladder might combine recordings from multiple roots, rather than rely on activity from a single root.

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