Proof of Concept

Final Report March 2016

Principal Investigator:	Prof Thelma Lovick
Co-Investigators:	
Title of Study:	**Pelvic nerve stimulation to control urinary incontinence**
Aims and Objectives: (max 400 words)	Urge Urinary urge incontinence (UUI) is defined as "a sudden compelling desire to pass urine, which is difficult to defer". UUI is rated as the most bothersome of lower urinary tract symptoms (Argawal et al, 2014). Prevalence rates of 13.3% for men and 30.0% for women have been reported in the UK, USA and Sweden (Milsom et al, 2014). The condition causes considerable distress to sufferers. Currently UUI is not well managed. The underlying pathology is not understood but may lie in the bladder (overactive bladder) or alternatively, the micturition control circuitry in the brain and/or spinal cord may be at fault. Urinary voiding is dependent on the integrated contraction of the detrusor muscle, and relaxation of the external urethral sphincter to permit urine to flow. These two events are controlled respectively the pelvic and pudendal nerves under the control of the central nervous system. Under normal circumstances, the central circuitry permits voiding to occur only when it is safe and socially acceptable for the individual to do so. In UUI this control appears to be lost. The aim of our study was to investigate in an animal model, whether high frequency electrical stimulation of the pelvic nerve, designed to produce a temporary reversible block of propagation of action potentials, could be used to prevent voiding from occurring even when the micturition threshold had been reached. We sought to produce an effect that was rapid in onset and offset, could be sustained for several minutes, was repeatable and produced minimal 'off target' effects on other organ systems. If the initial proof of concept experiments in anaesthetised rats proved successful, the next stage of the work would be to translate the findings to chronically instrumented conscious animals. References: Agarwal A, Eryuzlu LN, Cartwright R, Thorlund K, Tammela TL, Guyatt GH, Auvinen A, Tikkinen KA. (2014). What is the most bothersome lower urinary tract symptom? Individual- and population-level perspectives for both men and women. Eur Urol. 65:1211-1217. Milsom I, Coyne KS, Nicholson S, Kvasz M, Chen CI, Wein AJ. (2014) Global prevalence and economic burden of urgency urinary incontinence: a systematic review. Eur Urol. 65:79-95.

Description of research work: (max 400 words)	We used the urethane anaesthetised rat preparation in which continuous infusion of saline into the bladder (6ml h^{-1}) evokes repeated co-ordinated voids every few minutes (Crook and Lovick, 2016). Since the mechanics of voiding are essentially normal under urethane anaesthesia but volitional control is absent, the urethane-anaesthetised preparation approximates to the situation that characterizes urinary urge incontinence in humans. In the rat preparation we investigated whether unilateral high frequency stimulation of the preganglionic pelvic nerve bundle at stimulation parameters designed to block transmission of nerve activity, could prevent voiding from occurring, even when the bladder was filled up to the micturition threshold. We determined optimal stimulation parameters, whether the effect was repeatable and reversible, whether pelvic nerve stimulation produced 'off target' effects on the bowel, the uterus (in females) or abdominal wall and whether the stimulation evoked changes in blood pressure, heart rate and respiration. We also investigated whether the EMG activity of the external urethral sphincter was affected by pelvic nerve stimulation. The laparotomy performed to access the bladder and pelvic nerve, precluded measurement of pressure in the abdominal cavity. Therefore we measured EMG activity in the abdominal wall as a surrogate for intra-abdominal pressure changes.

Key findings:

1. High frequency pelvic nerve stimulation inhibits urinary voiding

Continuous infusion of saline into the bladder evoked repeated voids, which were characterised by a phasic rise in bladder pressure and the development of bursting activity in the external urethral sphincter as urine was expelled through the urethra, in agreement with our previous studies (Stone et al, 2011; Crook and Lovick 2016).

Unilateral stimulation of the preganglionic pelvic nerve bundle initiated within 1s of the onset of the sharp rise in bladder pressure signaling an imminent void, aborted the void and urinary continence was maintained. Using optimal stimulation parameters (see below) urinary continence could be maintained throughout the 1min period of stimulation despite continuing to infuse saline into the bladder (Figs 1A and 2). During this time there was an increase in tonic activity in the external urethral sphincter, but the bursting activity, which characterized voiding was not seen. Voiding usually resumed within a minute of switching off the stimulation (Figs 1A and 2). Importantly, stimulation applied during the filling phase in between voids had no effect, apart from small transient 'on' and 'off' fluctuations in bladder pressure (Fig 1B).

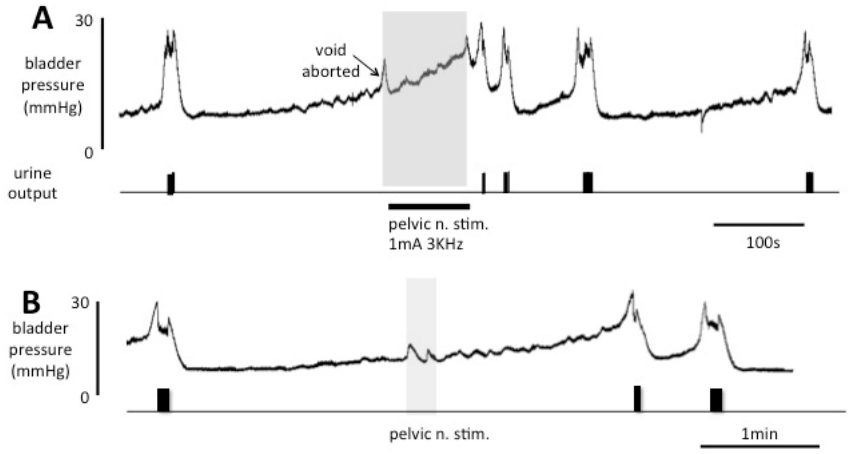

Fig1A. Voiding elicited by continuous infusion of saline into the bladder in a urethane anaesthetised rat. Initiating pelvic nerve stimulation (grey panel) at the onset of an imminent void indicated by a sharp rise in bladder pressure, aborted the void and urinary continence was maintained during the 1min period of stimulation, despite continued infusion of saline into the bladder. Voiding resumed once the stimulation was switched off. **B**. Pelvic nerve stimulation during the filling phase had little effect

2. Optimal stimulation parameters.

Optimal stimulation frequencies for blocking voiding using a sinusoidal waveform 1 -2mA intensity varied between 1-3KHz in different animals. Stimulation at suboptimal frequencies often resulted in the void being deferred until later in the 1min stimulation period, rather than being blocked for the duration of the stimulation. Alternatively, a void still occurred at the onset of stimulation but was of a much lower volume than during the pre-stimulation period (Fig 2A). The blocking effect on micturition was absent at stimulation frequencies >3-5KHz (Fig 2C). Low frequency stimulation (500Hz) was either ineffective or produced sub-optimal effects. Moreover, at these frequency, the stimulation often evoked changes in blood pressure, heart rate and respiration, as well as significant increases in bladder pressure (Figs 2A and 3A).

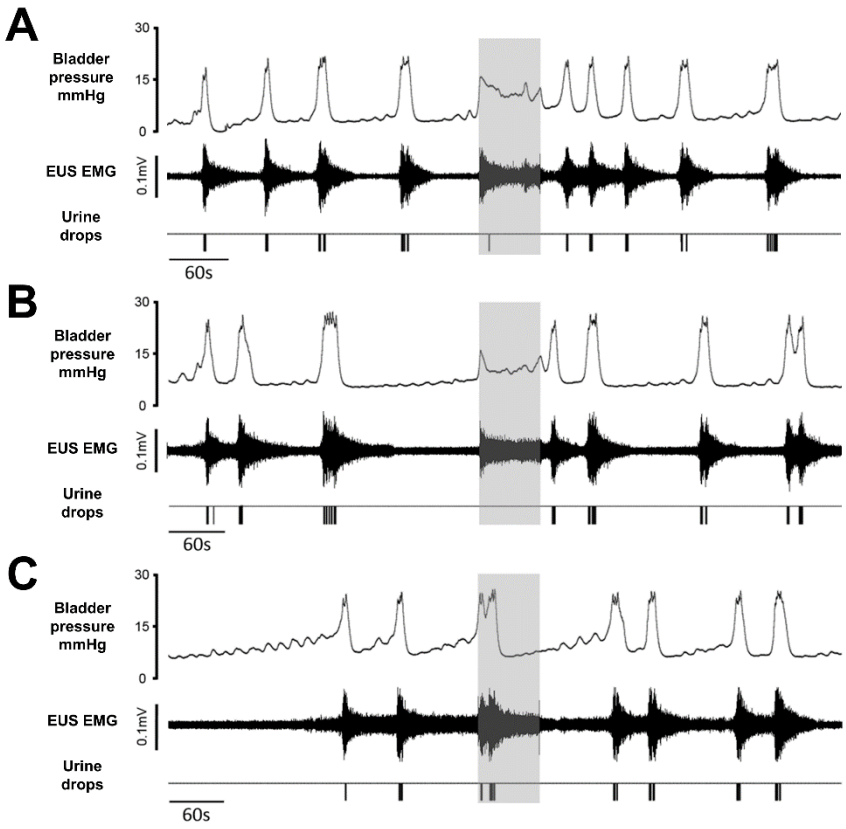

Fig 2A-C. Frequency dependent effect of 1min pelvic nerve stimulation on urinary voiding evoked by continuous infusion of saline into the bladder. Top trace of each set shows change in bladder pressure, middle trace shows EMG activity of the external urethral sphincter, lower trace shows timing of drops of urine expelled from the urethra. Grey panel indicates period of pelvic nerve stimulation. **A**. Low frequency stimulation (500Hz) did not completely inhibit voiding. **B**. Stimulation at 1KHz prevented voiding for the duration of the stimulation period. Voiding resumed within 30s of terminating the stimulus. **C**. Stimulation at 3KHz had no effect on voiding. All data from the same animal.

Unilateral v bilateral stimulation –

Stimulation of either the right or left pelvic nerve was equally effective in inhibiting voiding. Bilateral stimulation was no more effective in inhibiting voiding than unilateral stimulation.

3. 'Off target' effects

A number of 'off target' effects could be evoked by pelvic nerve stimulation. However, by adjusting the stimulation parameters, it was possible to inhibit micturition selectively with minimal 'off target' side effects.

i) Cardiorespiratory changes

In some animals pelvic nerve stimulation at the lower end of the effective frequency range for inhibiting or deferring voiding evoked a rise in blood pressure, tachycardia and an increase in respiratory rate (Fig 3A). However, by adjusting the stimulation parameters we were able to minimize or completely prevent these effects whilst still inhibiting voiding (Fig 3B).

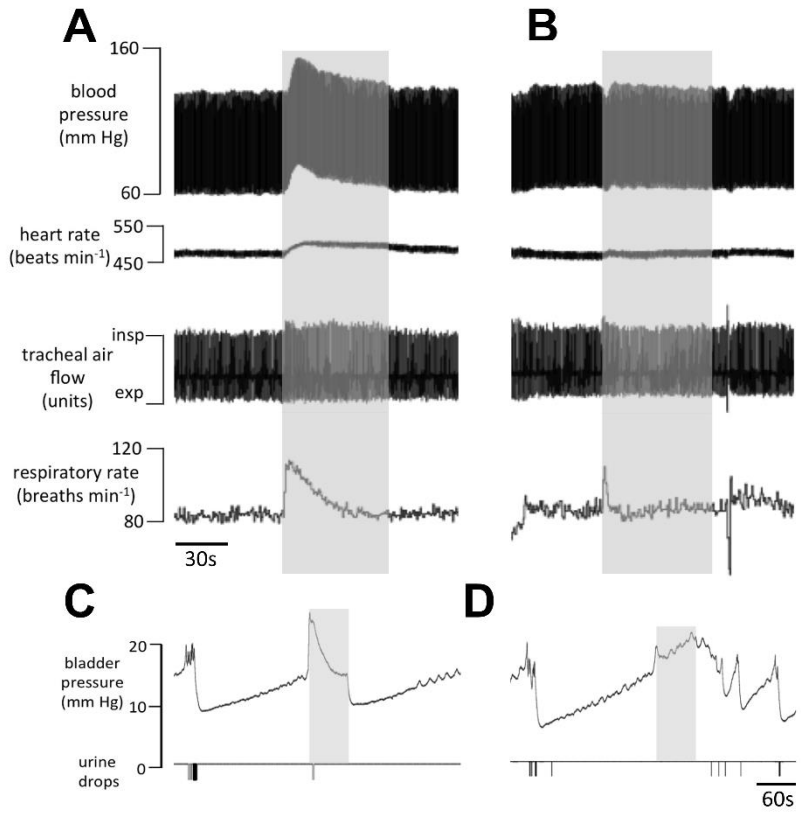

Fig 3A and B. Cardio-respiratory changes evoked by 1min sinusoidal pelvic nerve stimulation. **A** and **C** Stimulation at 500Hz evoked cardiorespiratory changes (A). At the same time bladder pressure rose (C) and voidinging was partially inhibited but bladder pressure rose considerably. **B** Stimulation at higher frequency (3KHz) in the same rat was without effect on blood pressure and heart rate whilst voiding was inhibited completely (D) There was a very short lasting (5s) tachypnea at the onset of stimulation.

ii) Changes in uterine and rectal pressure.

The pelvic nerve runs along the wall of the uterus in females; we were therefore concerned about the possibility of stimulus spread to the uterine muscle. We therefore monitored uterine pressure during stimulation. We found that pelvic nerve stimulation at frequencies that were effective in inhibiting voiding had no effect on intra-uterine pressure (Fig 4A). Similarly, there was no change in rectal pressure (Fig 4B).

iii) Changes in abdominal muscle EMG

Since a laparotomy had been performed to gain access to the bladder and pelvic nerve, it was not possible to measure intra-abdominal pressure in our preparation. We therefore measured EMG activity in the abdominal wall as an index of the abdominal muscle contraction that would be expected to give rise to a change in intra-abdominal pressure in the closed abdomen. Stimulation of the pelvic nerve using parameters that were optimal for blocking urinary voiding produced an initial brief contraction of the abdominal wall at the onset of the stimulation ('on response'; Fig 4C). Stimulation at lower-than-optimal frequency often evoked a more sustained contraction.

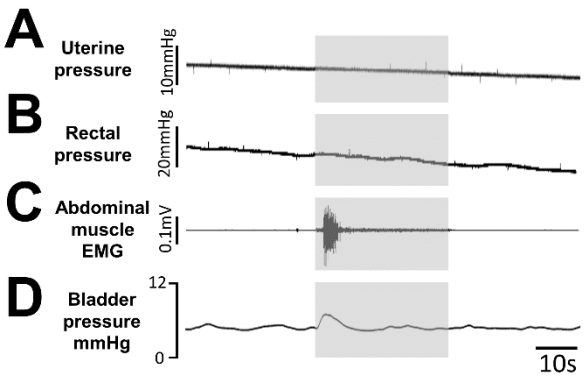

Fig 4A-D. Off-target effects of pelvic nerve stimulation (grey panels) using stimulation parameters optimal for inhibiting voiding (3KHz 1mA sinusoidal waveform), **A-B** pelvic nerve stimulation had no effect on uterine or rectal pressure. **C** transient contraction of abdominal wall following stimulus onset. **D** Pelvic nerve stimulation between voids evoked only small transient rise in bladder pressure at the onset of stimulation. All traces from the same animal.

Experiments in chronically instrumented rats

In our initial proposal to IMPRESS we had hoped in January 2016 to begin experiments on chronically instrumented animals. For this we required an amendment to the Home Office Project Licence, which covers this study. Despite having submitted the application in August 2015, we are still (April 2016) awaiting licence authority to begin these experiments. We have no reason to believe the amendment will not be granted in due course, but the process is extremely slow. We have not therefore been able to begin this work.

References:

Crook J, Lovick T (2016). Urodynamic function during sleep-like brain states in urethane anesthetized rats. Neuroscience. 313:73-82.

Stone E, Coote JH, Allard J, Lovick TA.(2011). GABAergic control of micturition within the periaqueductal grey matter of the male rat. J Physiol. 589: 2065-2078.

Outputs:

e.g. publications, new links etc.

Oral and poster presentations

1. Crook, JJ, Lovick TA 2015 Pelvic nerve stimulation to control urinary continence. Poster presented at the meeting at IMechE Nov 2015.

2. Crook JJ, Lovick TA Inhibition of urinary voiding by high frequency stimulation of the pelvic nerve. Abstract submitted for joint meeting of The Physiological Society and American Physiological Society, Dublin July 2016.

Public engagement

Crook JJ, Lovick TA. Pelvic nerve stimulation to control urinary continence. Poster presented at Bristol Festival of Neuroscience, March, 2016

New links – we have established a link with Professor Pedro Irazoqui, Director of the Centre for Implantable Devices, Purdue University, USA with a view to collaborating in the development miniature implantable devices using wireless power transfer to measure bladder pressure and deliver pelvic nerve stimulation in our rat model.

	We are also exploring the possibility of collaborating with colleagues in University of Aarhus, Denmark (Professor Karl-Erik Andersson) to translate our findings in rats to a porcine model, which will more closely model the human situation.

PROPOSED NEXT STEPS

Follow on funding Strategy:	Our proof of concept study in anaesthetised rats has demonstrated that high frequency stimulation of the pelvic nerve can exert a powerful block on urinary voiding. The effect is rapid in onset, repeatable and rapidly reversible and importantly, can be produced without significant 'off target' effects on other pelvic organs or the cardiovascular and respiratory systems. We believe that the technique shows great potential for development as a therapeutic intervention for intractable urge urinary incontinence in humans. We therefore propose to seek significant funding to continue the work, firstly in conscious instrumented rats and thereafter, using pigs as an intermediary translational stage to humans.
Future research work plan:	1. Consolidate our initial findings in the acute urethane anaesthetised rat model. 2. Work up a conscious rat model chronically implanted to record bladder pressure and to stimulate the pelvic nerve. A commercially available radiotelemetry device may be used to record pressure. Alternatively, a fluid filled catheter implanted through the bladder dome may be used for recording and infusion of fluid into the bladder via a tethered system. Since changes in abdominal pressure will be transmitted to the bladder and are likely to produce spurious effects, a second pressure sensor in the abdominal cavity will measure changes in abdominal pressure, which will then be subtracted electronically from the bladder pressure record to reveal true detrusor pressure. Stimulation will be carried out initially using a conventional tethered leads, moving to a wireless power transfer system (the Bionode from Purdue Univ) as the technology becomes available. "Urge" in the rat will be signaled by the sharp rise in bladder pressure that signals an imminent void. Pelvic nerve stimulation will be initiated at the onset of the rise in bladder pressure. Urine output will be monitored by placing the rat in a restraining tube with a urine collection cup for the duration of the experimental procedure. At a later stage rats may be freely moving in a metabolic cage in order to collect urine; alternatively the stimulation procedure may be carried out in freely moving rats in their home cages, the floor will be lined with ninhydrin-coated Strathmore paper to absorb urine spots, which then appear as a purple stain. 3.Translate findings in the rat to a pig model. For this stage we envisage collaborating with colleagues who have existing pig facilities and experience in using porcine models. We are already in discussions with potential collaborators in Denmark (University of Aarhus) who have considerable experience of working with pigs, as well as our bioengineering colleagues at Purdue University, USA, which has a pig facility.

We encourage you to use diagrams and figures to illustrate your work and you may also submit additional material such as videos.